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Biomedical Research Technology Resources

A Research Resources Directory

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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Revised
December 1984

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
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National Institutes of Health

NIH Publication No. 85-1430

Researcher uses laser microbeam instrument at the University of California, Irvine, to surgically alter a living cell, enabling analysis of the cell's function and the interrelation of the cell's components.

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Biomedical Research Technology Program

The Biomedical Research Technology Program concentrates on the application of the physical sciences, mathematics, and engineering to biology and medicine. Ultrasophisticated instruments, the latest state-of-the-art methods, and expert support personnel provided by the program help life scientists to decipher fundamental biological processes of the human body. The program's capabilities include large-scale and minicomputer systems; biochemical and biophysical instruments (mass spectrometers, nuclear magnetic resonance spectrometers, electron spin resonance spectrometers); million-volt electron microscopes; lasers; flow cytometers; vibrating probes; biomedical engineering technologies; and production of biochemical research materials.

The complex computer systems are used primarily for statistical data reduction, mathematical analyses, biomedical modeling, and organized knowledge systems. Other computers are used for specialized operations such as monitoring vital signs in critically ill patients. The biomedical and biophysical instruments facilitate research on biological structure and function at the molecular level. The biomedical materials resources produce labeled chemical compounds for use in research.

The Biomedical Research Technology Program works with biomedical scientists toward the development of new research technologies or new applications of existing technologies. In addition, the program provides training for the research community in the use of all biomedical technology tools and procedures.

The resources supported by the program are available for use by scientists with qualified projects. Scientists interested in using and obtaining information about a resource listed in this directory should contact the user contact person listed for that resource.

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Directory Organization

Title of Resource and Principal Investigator

The directory entries are organized by type of resource and, within each type, resources are arranged alphabetically by title. A geographical index is provided at the end of the directory. In it, available resources are listed alphabetically by state and alphabetically by type of resource and by title within each state. The index provides the names of the cities in which the facilities are located and the page on which each directory entry appears.

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Title of Resource and Principal Investigator

Resource:
National Stable Isotopes Resource (SIR) at Los Alamos

Principal Investigator:
James A. Fee, Ph.D.
INC-4, MS-C346
Los Alamos National Laboratory
Los Alamos, New Mexico 87545
(505) 667-6045/5324

Services Available

- *Chemical Materials:* Separated ^{13}C, ^{15}N, and ^{18}O; labeled compounds not available commercially, upon request.
- *Service Personnel:* Collaboration, including provision as necessary of isotope-labeled compounds and analysis and data interpretation with NMR and isotope-ratio MS; advice and assistance in use of isotopes; and training in use of stable isotopes.
- *User Laboratory Space:* Resource hosts visiting scientists and postdoctoral fellows and encourages exchanges of talks, short courses, and extended research opportunities. Separate user space is provided.
- *User Contact Person:* James A. Fee, Ph.D. (505) 667-6045/5324.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The focus of this resource is on developing the utility of stable isotopes in biology and medicine by:

Producing and providing enough separated ^{13}C, ^{15}N, and ^{18}O to support the program; synthesizing labeled compounds for accredited investigators when those compounds are not readily available from commercial or other sources at acceptable costs; developing an active program of research collaboration with investigators in the biosciences community, providing, as necessary, isotope-labeled compounds and analysis and data interpretation with NMR and MS;

Advising and assisting investigators using isotopes of carbon, oxygen, and nitrogen;

Hosting visiting scientists for training in use of stable isotopes and encouraging exchanges of talks, short courses, and extended research opportunities; and

Collaborating with other resources in synthesis and MS and NMR analyses to speed development of isotope methodology.

- *Current Research:* Biosynthesis of natural products, especially L-amino acids labeled with ^{13}C, ^{15}N, ^{17}O, and/or ^{18}O; development of improved chemical syntheses for labeled metabolic and biosynthetic precursors; study of intermediary metabolism in intact cells, tissues, and perfused organs using ^{13}C labeled precursors and NMR detection; structural studies of lipids, proteins, and other components of intact cells enriched with the stable isotopes of carbon, nitrogen, and oxygen.
- *New Concept/Capability:* These stable, nonradioactive isotopes are being used in human nutrition and pharmacokinetic studies and in clinical research without the delivery of radiation burden to the subject. They have been incorporated into specific sites of enzymes and nucleic acids and, through their characteristic NMR signatures, have provided unique regiospecific information about the structure, dynamics, and function of these macromolecules.

[1]Walker, T. E., Han, C. H., Kollman, V. H., London, R. E., and Matwiyoff, N. A., *Journal of Biological Chemistry* 257:1189, 1982.
[2]Unkefer, C. J., London, R. E., Whaley, T. W., and Daub, G. H., *Journal of the American Chemical Society* 105:733, 1983.
[3]Matwiyoff, N. A., London, R. E, and Hutson, J. Y. in *NMR Spectroscopy: New Methods and Applications*, ACS Symposium Series 191, 1982, pp. 157-186.
[4]London, R. E., Groff, J. P., Cocco, L., and Blakley, R. L., *Biochemistry* 21:4450, 1982.

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Title of Resource and Principal Investigator

Resource:
National Tritium Labeling Facility

Principal Investigator:
Henry Rapoport, Ph.D.
Lawrence Berkeley Laboratory
University of California, Bldg. 3
Berkeley, California 94720
(415) 486-4325

Services Available

- *Instruments:* Bruker 270 MHz NMR with tritium probe; one preparative and one analytical Waters HPLC; Hewlett-Packard 1040A high-speed photodiode detector system with printer/plotter/integrator/floppy disks; Berchtold radioactivity flow monitor for HPLC; Varian 3700 GLC with heated proportional flow counter and associated electronics for multiscaling analysis; and LKB fraction collector with automatic slope sensing.
- *Special Features:* The laboratory operates as a research, teaching, and tritium-labeling facility. Outside researchers may use the facility under supervision and with adherence to strict safety precautions. The resource contains instruments and techniques that are required for labeled compounds of a high degree of radiochemical purity. Tritium NMR and gas-liquid radiochromatography are combined to determine the tritiated positions and the number of tritons per molecule.
- *Service Personnel:* Qualified investigators use the facility under the supervision of the resident staff.
- *User Laboratory Space:* Chemical bench and desk space are provided for users while they are visiting the laboratory.
- *User Contact Persons:* Raymond Aune, (415) 486-5251; Benjamin Gordon (415) 486-4337; Hiromi Morimoto, (415) 486-4337; Wallace Erwin, (415) 486-4337.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Mechanisms by which tritium reactions take place; number and positions of tritons in a labeled molecule; and degree of radiochemical purity.
- *Current Research:* Reaction mechanisms in the microwave discharge method of tritium labeling; development of dehalogenation procedures for specific labeling; investigations of the retention-time shift of tritium-labeled molecules by gas-liquid radiochromatography; and improving T-NMR sensitivity.
- *New Concept/Capability:* The facility provides to biomedical researchers commercially unavailable, very-high, specific-activity tritium-labeled compounds, at a high degree of radiochemical purity. The resource aim also is to increase knowledge of the mechanisms of tritiation reactions.

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Biological Structure and Function: Biomedical Kinetics

Title of Resource and Principal Investigator

Resource:
Center for Fast Kinetics Research

Principal Investigator:
E. Lawrence Powers, Ph.D.
University of Texas, Austin
Austin, Texas 78712
(512) 471-4615

Services Available

• *Electron Pulse Radiolysis Systems:* (1) 5.5 MV HVEC CN Van de Graaff accelerator; up to 1 ampere peak current at pulse widths 30-800 ns. Kinetic spectrometer (absorption and emission) with nanosecond time resolution; wavelength range, 220-2,400 nm. Kinetic conductivity with microsecond resolution. (2) 4.0 MV HVEC KN Van de Graaff accelerator; up to 5 A peak current in pulse widths 12 ns to 1μs. Diagnostic facilities available as for (1) supplemented by resonance Raman spectroscopy with ns time resolution.

• *Laser Photolysis System:* (1) Q-switched Nd:YAG oscillator-plus-amplifier (Quantel YAG 481) giving about 11 ns pulses of 900 mJ at 1064 nm. Harmonic generators provide 330 mJ at 532 nm, 120 mJ at 355 nm, and 75 mJ at 266 nm at 10 Hz repetition. (2) Quantel TDL III dye laser pumped by (1). Produces tunable radiation in visible and with frequency doubling, in ultraviolet range. Maximum output energy about 120 mJ per pulse at 590 nm. (3) Hydrogen-filled (100 psi) Raman-shift cell pumped by (1) to produce discrete lines in Stokes, and anti-Stokes, regions. Items (1) to (3) are coupled to nanosecond kinetic spectrophotometer system similar to electron pulse system.

• *Picosecond Spectroscopy System:* Mode-locked Nd:YAG oscillator plus two amplifiers giving pulses (60 mJ at 1064 nm; 30 ps FWHM or 200 mJ at 1064 nm; 200 ps FWHM) with rep-

Continued on next page

Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* This resource provides the capability of initiating and detecting very fast chemical and photophysical processes involving biological molecules, membranes, and cells. The electron pulse radiolysis facility is capable of generating hydrated electrons and hydroxyl radicals in aqueous solution, making possible the study of a wide range of free radical chemistry pertinent in chemical and biological systems. In nonpolar solutions, the electron beam produces electronically excited states of the solute molecules, providing an opportunity to study energy transfer and to measure triplet-triplet absorption spectra. The laser flash photolysis and photon-counting systems are capable of exciting fluorescence probes and measuring lifetimes as short as 5×10^{-11} seconds. They are also useful for a wide variety of photophysical and photochemical studies, such as the primary processes in vision and the generation of singlet oxygen.

• *Current Research:* System development; reactions of DNA with OH radicals; chemical basis for sensitization of cells to radiation;[1] dose rate effects in irradiated cells; electron transfer at water-lipid interfaces; fluorescence study of ribosomal structure;[2] Raman spectroscopy of short-lived species; singlet oxygen in microheterogeneous systems; photophysics of visual pigments;[3] photoproperties of porphyrins;[4] reactivities of organic biradicals; fluorescence probe studies of DNA; and the radiation chemistry of free radical traps.[5] In addition, approximately thirty different programs are currently in progress in areas of biomedical relevance.

• *New Concept/Capability:* The possibility exists for performing precise measurements of the lifetime and rotational times (*via* time-resolved emission anisotropy) of fluorescent

Continued on next page

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Services Available

etition rates up to 10 Hz. Harmonic generators producing 265, 353, and 530 nm or with Raman shift cell (sec 3 above). Diagnostics by ultrafast photodiode and photomultiplier tube (subnanosecond and upward) by streak camera (10 ps to 4 ns), or by diode array spectrograph.

- *Time-Correlated Single Photon Counting:* Synchronously pumped, mode-locked, cavity-dumped dye laser tunable in range 560 nm to 780 nm (280-390 nm after frequency-doubling). Fluorescence lifetime and emission anisotropy by time-correlated single photon counting. Lifetimes near 100 ps resolvable.
- *Electron-Photon Double-Pulse Facility:* Pulsed electron beams and pulsed laser beams can be used to irradiate target systems either simultaneously or delayed, one with respect to the other. Diagnostics by kinetic spectrometry.
- *Infrared Luminescence:* Germanium photodiode and amplifier assembly for detecting low level emission in range 1100-1600 nm, approximately 1μs time resolution.
- *Spectrofluorometer:* (1) Modular design for simultaneous observation of parallel and perpendicular components of polarized light for anisotropy studies. Controlled by PET microcomputer; (2) commercial spectrofluorometer for recording excitation and emission (phosphorescence or fluorescence) spectra.

Continued on next page

Research Emphasis or Application

probes attached to proteins (and ribosomes), embedded in membranes or complexed with DNA. Time scales in the range 10^{-11} to 10^{-7} seconds can be probed. These make available information on molecular/segmental motion, structural dynamics, organelle structure, etc. The use of Raman techniques allows vibrational spectra of short-lived free radicals and electronically excited states to be obtained.

[1]Beaumont, P. C., and Powers, E. L., *International Journal of Radiation Biology and Related Studies in Physics, Chemistry, and Medicine* 43:485, 1983.

[2]Odom, O. W., Jr., Robbins, D. J., Lynch, J., Dottavio-Martin, D., Kramer, G., and Hardesty, B. A., *Biochemistry* 19:5947, 1980.

[3]Becker, R. S., Freedman, K., and Causey, G., *Journal of the American Chemical Society* 104:5797, 1982.

[4]Reddie, E., Jori, G., Rodgers, M. A. J., and Spikes, J. D., *Photochemistry and Photobiology* 38:639, 1983.

[5]Sridhar, R., Beaumont, P. C., and Powers, E. L., *Radiation Research* 94:600, 1983.

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Services Available

- *Stopped-Flow Assembly:* Durrum fast-mixing assembly coupled to kinetic spectrometer. Two or three solutions can be mixed (millisecond resolution). Can be used with electron pulse generators and laser systems.
- *Computer System:* Facilities listed above are on-line to a dedicated PDP-11/70 computer via appropriate fast digitizers. Data collection, reduction, analysis, and print-plot together with experiment control; concurrent operation from several graphics terminals.
- *Service Personnel:* Resource personnel perform all system operations.
- *User Laboratory Space:* For routine operations.
- *User Contact Person:* M. A. J. Rodgers, Ph.D., or S. J. Atherton, Ph.D., (512) 471-7583.

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Name of Resource and Principal Investigator

Resource:
University of Pennsylvania Regional Laser Laboratories

Principal Investigator:
Robin M. Hochstrasser
Director, RLBL
Department of Chemistry
University of Pennsylvania
Philadelphia, Pennsylvania 19104
(215) 898-8410

Services Available

- *Instruments:* Subnanosecond fluorescence and Raman spectrometers using pulsed and CW mode-locked YAG lasers.
- *Special Features:* Instruments, now largely constructed, can rapidly record fluorescence and Raman spectra, timegated in the range of 15 ps to many ns. The 0.5 ps capability is still under construction. Facilities will be available for pump-probe experiments using a variety of optical wavelengths. The longer time needs (>300 ps) are met by time-correlated photon counting techniques, and upconversion methods are used to obtain accurate fluorescence decays on the time scale t>10 ps with a mode-locked, Q-switched CW YAG laser. Raman spectra are being obtained for t>10 ps by means of a 20 Hz pulsed YAG system, while for t≥0.5 ps a CW mode-locked YAG laser will synchronously pump a dye laser.
- *Service Personnel:* Technical personnel are available to assist users in performing experiments, to consult on research problems, and to work out details of experimental planning.
- *User Laboratory Space:* Some office space is provided. Users are expected to be on-site and sufficient space is available around the instrumentation to enable user involvement with apparatus and sample manipulation. Wet lab, cells, vacuum system, and, at additional cost, NMR and mass spectrometer are available.
- *User Contact Persons:* Dr. Gary Holtom, head of laser operations, (215) 898-5891; Helen Sewack, secretary (215) 898-4294.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource allows the acquisition of fluorescence and Raman spectra, timegated fluorescence and Raman spectra, kinetic data using fluorescence and Raman probes, fluorescence lifetimes, high S/N spontaneous decay processes suitable for deconvolution, time-evolution of fluorescence depolarization, and time-evolution of Raman anisotrophy.
- *Current Research:* Development of instrumentation to study prototype ultrafast processes in liquids, molecular relaxation, and conformational change; polyene dynamics; heme-protein dynamics; electron transfer processes in reaction centers; molecular motion in proteins (for example, tryptophan); hemoglobin cooperativity; and fluorescence of aromatic amino acids.
- *New Concept/Capability:* Ultrafast fluorescence, 100 ns ≥t≥0.5 ps, and Raman resource having entirely unique capabilities. The instruments are being designed and developed in-house specifically for general use with biomedical research problems.

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Title of Resource and Principal Investigator

Resource:
Laser Microbeam Program (LAMP)

Principal Investigator:
Michael W. Berns, Ph.D.
School of Biological Sciences and College of Medicine
Beckman Laser Institute and Medical Clinic
University of California, Irvine
Irvine, California 92717
(714) 856-6291

Services Available

- *Instruments:* Four different laser microbeam instruments; YAG pumped-dye laser tunable between 220 and 700 nm with power output up to 10^5 W, pulse duration of 10 ns, attached to a Zeiss axiomat microscope and an LSI 11/23 driven image array processing computer; YAG picosecond laser with pulses to 25 ps, power to 10^9 W, wavelengths of 1.06 microns and 532, 355, and 265 nm, attached to axiomat and image array computer; YAG dye laser with output of 13 kW at 532 and 265 nm, combined with Zeiss photo microscope III; laser fluorescence photobleaching and quantitative fluorescence system employing a helium-cadmium laser at 325 or 441 nm, interfaced with a Tracor Northern multichannel analyzer scanning array diode and microspectrofluorometer.
- *Special Features:* Laser can be focused to less than 1 micrometer spots into living or dead materials viewed under a microscope; images can be simultaneously manipulated and enhanced by the computer; microscope has digital-controlled stage and automatic focusing device; accessory capabilities in electron microscopy and cell cultures are available.
- *Service Personnel:* Resource personnel maintain and operate the microbeam and the computer with the users; user often can be allowed to operate equipment after initial training; special 5-day minicourse given yearly to train potential users.
- *User Laboratory Space:* Small work area provided for users; special arrangements for tissue culture and animal care possible.
- *User Contact Person:* Michael W. Berns, Ph.D., (714) 833-6291.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The laser microbeam is able to focus an intense laser beam of wavelength between 200 and 700 nanometers to a lesion-producing spot as small as 0.1 micrometer in diameter inside living or fixed cells and tissues. By choosing appropriate wavelengths that match specific natural or applied chromophores, it is possible to selectively alter a small part of an organelle or even to disrupt a specific molecular component of that organelle. Furthermore, by employing the nonlinear effects of short-pulsed, high-intensity lasers, it is possible to disrupt subcellular regions via uncommon physical phenomena such as multiphoton absorption, dielectric breakdown, acoustic effects, etc. All of the above alterations can be achieved in localized subcellular regions while maintaining cell viability. The cells are simultaneously observed and their images enhanced by using a specially designed image array computer. Capabilities also exist to use the lasers to stimulate fluorescence and record changes in fluorescence over time from a few nanoseconds after irradiation. This capability can be used to study oscillating fluorescence reactions and photobleaching recovery of the cell surface.
- *Current Research:* Laser microirradiation of mitotic organelles such as centrioles, kinetochores, and microtubules and subsequent analysis of cell behavior and ultrastructure; laser microirradiation of mitochondria in muscle cells and subsequent analysis of contractility and membrane electrical properties; laser microirradiation of cytoplasmic stress fibers, myofibrils, and bands of 100 A filaments; laser microablation of portions of imaginal disks in *Drosophila* larvae and subsequent effects on pattern formation; laser ablation of the neurosensory appendages of insects during development and the subsequent effects in developmental neurobiology; laser-induced fluorescence in subcellular compartments such as mitochondria and chromosomes; and laser-induced photobleaching and photobleaching recovery in cell membranes. Laser interactions with tissues and cells as they relate to clinical applications are now emphasized.
- *New Concept/Capability:* The ability to selectively alter subcellular regions as small as 0.1 micrometer is providing the capability to define the structure and function of cellular organelles from an entirely new perspective. For example,

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Research Emphasis or Application

it has been possible to selectively damage different regions of the centrosome and demonstrate that it has subcellular molecular organization in different regions and that these regions control different aspects of cell division. Using natural absorbency as well as absorbency induced by psoralens, acridine orange, and BUDR, it has been shown that an RNA in the pericentriolar cloud is responsible for controlling microtubule organization and chromosome movement. This approach can now be applied to other problems in cell motility [illegible] just barely [illegible]

[illegible] is greatly [illegible] because [illegible] enhance the live [illegible] is greatly [illegible] irradiation of subcellular [illegible] fuzzy or not [illegible] light microscopic analysis is also greatly [illegible] through further image enhancement as well as computer-controlled trabbing of the irradiated structures and/or cell. The laser microbeam is also extending the microablation technique into experimental embryology where small regions inside living embryos or larvae can be altered without disturbing surrounding embryonic tissues. This very fine microsurgical capability does not involve physically touching or disrupting the embryo by the investigator.

[1]Berns, M. W., Aist, J., Edwards, J., Strahs, K., Girton, J., Kitzes, M., Hammer-Wilson, M., Liaw, L.-H., Siemens, A., Koonce, M., Walter, R., van Dyk, D., Coulombe, J., Cahill, T., and Berns, G. S., Laser microsurgery in cell and developmental biology. *Science* 213:505-513, 1981.

[2]Siemens, A., Walter, R. J., Liaw, L-H., and Berns, M. W., Laser stimulated fluorescence of submicron regions within single mitochondria of rhodamine-treated myocardial cells in culture. *Proceedings of the National Academy of Sciences* 79:466-470, 1982.

[3]Aist, J. R., and Berns, M. W., Mechanics of chromosome separation during mitosis in *Fusarium* (Fungi Imperfecti): New evidence from ultrastructural and laser microbeam experiments. *Journal of Cell Biology* 91:446-458,1981.

[4]Walter, R. J., and Berns, M. W., Computer-enhanced video microscopy: Digitally processed microscopic images can be produced in real-time. *Proceedings of the National Academy of Sciences* 78:6927-6931, 1981

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Biological Structure and Function: Biostructure

Title of Resource and Principal Investigator

Resource:
National Flow Cytometry Resource

Principal Investigator:
L. Scott Cram, Ph.D.
Experimental Pathology Group
LS-4, MS M888
Los Alamos National Laboratory
Los Alamos, New Mexico 87545
(505) 667-2785

Coinvestigators:
Marty F. Bartholdi, Ph.D.
Harry Crissman, Ph.D.
James Jett, Ph.D.
John Martin, B.A.
Gary Salzman, Ph.D.
John Steinkamp, Ph.D.

Services Available

- *Instruments:* Two multiwavelength cell sorters equipped with two or three lasers for sequential excitation (UV-argon, krypton, visible-argon) including one with high-speed sorting capability; a modified FACS II cell sorter; an EPICS V cell sorter (dual laser); a chromosome high-resolution imaging sorter; epi-illumination fluorescence microscope and spectrophotofluorometer for sample preparation; and computers for data analysis.
- *Special Features:* List-mode data acquisition of up to eight variables per cell, axial light-loss corrected for variation in illumination intensity, multicolor fluorescence measurements, fluorescence polarization analysis, fast kinetic measurements, DC Coulter volume, multiangle light scatter measurements, high illumination irradiance of chromosomes; offline data analysis using the Los Alamos central computing facility.
- *Service Personnel:* Resource personnel available to assist in experimental design, sample preparation, analysis, and data reduction. Staff members are available to implement new measurement techniques and to aid in data interpretation.
- *User Laboratory Space:* User offices and separate well-equipped tissue culture and sample preparation laboratories provided. Animal care facilities available on special request.
- *User Contact Persons:* L. Scott Cram, Ph.D., (505) 667-2785/2750; Gayle Travis, Resource Manager, (505) 667-2836.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Flow cytometry makes use of electro-optical techniques to provide quantitative analyses of many cell properties, for example, DNA, RNA, protein, and cell surface antigens. On the basis of these measured properties, subpopulations of cells may be separated for use in various biological studies. Cells or subcellular constituents, such as chromosomes, are individually analyzed and sorted at rates of up to a few thousand per second. Unique capabilities exist in the following areas: high-resolution chromosome analysis, multilaser excitation systems, axial light-loss measurements, kinetic measurements, and correlated data analysis procedures. Expertise has been developed in fluorescent staining, multivariate data collection and analysis, and sorting procedures.
- *Current Research:* Flow karyotype analysis of cells undergoing spontaneous neoplastic transformation; chromosome sorting and construction of human DNA sequence libraries; correlated determination of enzyme mass and enzyme activity; quantitative analysis and sorting of the various host cell types and tumor cells in heterogenous tumor population; low-level mutagen effects on cells and chromosomes; proliferation and differentiation of mononuclear phagocytes; and lung damage and inflammatory response.
- *New Concept/Capability:* The ability to quantitate multiple properties of individual cells and chromosomes at the rate of 1,000 per second and to sort subpopulations based on these measurements has provided a new perspective to understanding heterogenous cell populations and detecting rare cell types. For example, a deletion in chromosome 13 has been linked with the occurrence of retinoblastoma. A Chinese hamster-human hybrid cell line that retained the normal human 13 chromosome was used as a source of chromosomes for sorting. Genomic libraries are being constructed from the sorted chromosomes. This approach

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Research Emphasis or Application

can be applied to many problems in cell and molecular biology. The addition of new capabilities for high-speed cell and chromosome sorting, sorting based on chromosome morphology, high-resolution analysis, and statistical analysis of eight parameter correlated data is now being used to extend the applications of flow cytometry.

[1]Cram, L. S., Bartholdi, M. F., Ray, F. A., Travis, G. L., Kraemer, P. M., Spontaneous neoplastic changes of Chinese hamster cells in culture: Multistep progression of karyotype. *Cancer Research* 43:4828, 1983.

[2]Standefer, J. C., Anderson, R. E., Wilder, M. E., Martin, J. C., Interactions of fluorescent probes with lymphocytes: Effects of irradiation. *American Journal of Pathology* 144:301, 1984.

[3]Van den Engh, G., Trask, B., Cram, L. S., Bartholdi, M. F., Preparation of chromosome suspensions for flow cytometry. *Cytometry* 5:108, 1984.

[4]Griffith, J. K., Cram, L. S., Crawford, B. D., Jackson, P. J., Schilling, J., Schimke, R. T., Walters, R. A., Wilder, M. E., Jett, J. H., Construction and analysis of DNA sequence libraries from flow-sorted chromosomes: Practical and theoretical considerations. *Nucleic Acids Research* 12:4019, 1984.

[5]Steinkamp, J. A., Stewart, C. C., and Crissman, H. A., Three-color fluorescence measurements on single cells excited at three laser wavelengths. *Cytometry* 2:226, 1982.

[6]Crissman, H. A., and Steinkamp, J. A., Rapid one-step staining procedures for analysis of cellular DNA and protein by single- and dual-laser flow cytometry. *Cytometry* 3:84, 1982.

[7]Hiebert, R. D., Jett, J. H., and Salzman, G. C., Modular electronics for flow cytometry and sorting: The LACEL system. *Cytometry* 1:337, 1981.

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Title of Resource and Principal Investigator

Resource:
National Vibrating Probe Facility

Principal Investigator:
Lionel F. Jaffe, Ph.D.
Marine Biological Laboratory
Woods Hole, Massachusetts 02543
(617) 548-3705

Services Available

- *Instruments:* Three vibrating probe systems, each with a probe that can be vibrated in a circular path. Such "two-dimensional" probes allow simultaneous measurement of two components of the electrical field, or current, near a living cell or multicellular system. Probes are used to explore the fields near specimens observed under a Zeiss inverted microscope. These images also appear on a video monitor. An associated computer immediately converts each field measurement into a vector superimposed upon the video image. These compound images are recorded. Two of the systems are supported on Newport air tables and are supplemented with equipment, allowing simultaneous measurement and/or voltage clamp with intracellular microelectrodes in combination with vibrating probe measurements.
- *Special Features:* A range of probe tip sizes as small as a few microns will be available. Two of the microscopes are equipped with Nomarski optics. One is equipped with epifluorescent optics.
- *Service Personnel:* Resource personnel maintain the systems and train users to operate the systems.
- *User Laboratory Space:* A small work area is provided for users in the facility. Larger work areas can be rented from the Marine Biological Laboratory (MBL) for specimen preparation and other accessory needs. Two-dimensional vibrating probes can be loaned to users for work at the MBL but outside the facility proper.
- *User Contact Person:* Carl Scheffey, Ph.D., (617) 548-3705 x271.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* With the vibrating probe the patterns of current driven by living systems can be explored, mapped, and measured. The instruments are useful particularly for measuring relatively steady currents, although the method can be used with a response time better than 100 msec. Since the probe is placed outside the living system, the latter is normally undisturbed by the measurement process. By systematically changing the composition of the medium and observing the resultant changes in current, one can study the specific ionic composition of these currents. The vibrating probe has been particularly useful in investigating currents involved in normal growth and development[4] as well as wound healing and regeneration.[2] It also has proven valuable in investigating complex transporting epithelia[5] and cell motility, as well as neuromuscular function.[1]
- *Current Research:* Studies of currents through gliding *Cyanobacteria*, growing *Acetabularia*, budding moss filaments, *Limulus* photoreceptor cells, cockroach oocytes, snake muscle spindles, and toad skin. Projects planned for the near future include corn roots, *Dictyostelium* slugs, ascidian eggs, squid eggs and nerve growth cones in culture. Two-dimensional vibrating probe usage is relatively new[3] and is still being perfected. We plan to develop a three-dimensional vibrating probe, an aerial probe, and a probe that may be inserted inside living organisms.

[1]Betz, W. J., Caldwell, J. H., Ribchester, R. R., Robinson, K. R., and Stump, R. F., Endogenous electric field around muscle fibers depends on the Na^+-K^+ pump. *Nature* 287:235-237, 1980.
[2]Borgens, R. B., What is the role of naturally produced electric current in vertebrate regeneration and healing? *International Review of Cytology* 76:245-298, 1982.
[3]Freeman, J. A., Weiss, J. M., Snipes, G. J., Mayes, B., and Norden, J. J., Growth cones of goldfish retinal neurites generate DC currents. *Neuroscience Abstracts* 7:550, 1981.
[4]Jaffe, L. F., Developmental currents, voltages, and gradients. In *Developmental Order: Its Origin and Regulation. Symposium Society of Developmental Biology* 40:183-215 (Subtelny, S., and Green, P. B., eds.) New York, N.Y., Alan R. Liss, 1982.
[5]Scheffey, C., Foskett, J. K. and Machen, T. E., Localization of ionic pathways in the teleost opercular membrane by extracellular recording with a vibrating probe. *Journal of Membrane Biology* 75:193-203, 1983.

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Title of Resource and Principal Investigator

Resource:
Collaborations on Mitosis Studies Using the Albany HVEM Resource

Principal Investigator:
Conly L. Rieder, Ph.D.
Division of Clinical Sciences
Wadsworth Center for Laboratories and Research
New York State Department of Health
Albany, New York 12201
(518) 474-2560

Coinvestigator:
Samuel S. Bowser, Ph.D.
(518) 474-2560

Services Available

- *Instruments:* Share instrumentation with the Albany High Voltage Electron Microscope Biological Facility for the East. This facility also contains a Nikon Optiphot phase-contrast Hoffman modulation-contrast/epifluorescence light microscope equipped with a UFX-35 mm automatic exposure system, and a Nikon Diaphot (invert) phase-contrast light microscope with an HFX-25 mm automatic exposure system. Either of these microscopes can be coupled to a Dage-MTI Model 65 video camera for real-time or time-lapse recordings on an NEC-Model VC-9507 videocassette recorder. A variety of microtomes are available including a Sorvall MT-2B, Reichert OmU-2, and four LKB-3's. Reconstruction from serial sections can utilize existing state-of-the-art computer hardware and software systems shared with the Albany Computer Facility for Three-Dimensional Reconstruction of Subcellular Structure.
- *Special Features:* Same as those at the Albany High Voltage Electron Microscope Biological Facility for the East.
- *Service Personnel:* Technical help is available for embedding, sectioning, staining, and examining the material. A postdoctoral fellow is available to assist in designing experiments to optimize HVEM potential, conduct preliminary feasibility studies, troubleshoot, and collect and analyze data.

Continued on next page

Research Emphasis or Application

- *Current Research:* The emphasis of this facility is on applying HVEM methods to collaborative studies involving mitosis. To this end, proposals are welcomed from investigators working on unusual organisms, the cells of which undergo an atypical mitosis, or from those investigators who are studying aspects of mitosis amenable to HVEM. Semithick to thick serial sections[1] are combined with new HVEM approaches[2,3] to answer various ultrastructural questions. Recent experiments have compared the three-dimensional spindle organization in mammalian,[4] insect,[5] and other organisms; we have also studied the role of the centrosome and kinetochore in spindle formation and function. This approach is especially suitable for those studies that utilize eggs[5,7] or other large cells, or those studies that require three-dimensional information on various mitotic components, including spindle-associated membranes, centrioles,[6,7] and/or microtubules.[4]

[1]Rieder, C. L., Rupp, G., and Bowser, S. S., *Journal of Electron Microscopic Technique*, In press.
[2]Rieder, C. L. and Bowser, S. S., *Journal of Microscopy* 132:71-80, 1983.
[3]Rieder, C. L. and Bowser, S. S., *Journal of Histochemistry and Cytochemistry*, In press.
[4]Rieder, C. L., *Chromosoma* 72:145-158, 1981.
[5]Rieder, C. L. and R. Nowogrodzki, *Journal of Cell Biology* 97:1144-1155, 1983.
[6]Rieder, C. L. and Borisy, G. G., *Biology of the Cell* 44:117-132, 1982.
[7]Sluder, G. and Rieder, C. L., *Journal of Cell Biology*, In press.
[8]Rieder, C. L., *International Review of Cytology* 79:1-58, 1982.

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Biological Structure and Function: Electron Microscopy

Services Available

- *User Laboratory Space:* Ample desk and specimen preparation space is available. This includes space for tissue culture, biochemical preparations, specimen sectioning and screening, video-light microscopy, and darkroom development, and computer facilities.
- *User Contact Person:* Conly L. Rieder, Ph.D., (518) 474-2560.

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Title of Resource and Principal Investigator

Resource:
Computer Facility for Three-Dimensional Reconstruction of Subcellular Structures

Principal Investigator:
Joachim Frank, Ph.D.
New York State Department of Health
Wadsworth Center for Laboratories and Research
Empire State Plaza
Albany, New York 12202
(518) 474-7002

Coinvestigators:
Conly L. Rieder, Ph.D.
James N. Turner, Ph.D.
(518) 474-6343

Services Available

- *Instruments:* Share instrumentation with the Albany High-Voltage Electron Microscope Biological Facility for the East. Additional instrumentation as follows:
- *Hardware:* DEC VAX 11/750 with RA 81 450 Mbyte disk unit and TU 80 tape drive; Lexidata Solidview/Image view terminal; AED 512 graphics terminal; AED 512 graphics terminal; Tektronix digital plotter; Perkin Elmer PDS 1010A flatbed microdensitometer with film-write option; optical diffractometer for prescanning electron micrographs.
- *Software:* SPIDER electron image processing software, including programs for three-dimensional (3-D) reconstruction using ART and modified back projection. MOVIE. BYU Display Software System for extracting polygon coordinates and displaying shaded surfaces.
- *Special Features:* The software programs allow double-tilt or conical tilt data from the high-voltage electron microscope (HVEM) to be used for reconstructing subcellular structures in three dimensions. A specialized high-precision tilt/rotation goniometer for the HVEM is being constructed. The use of double-tilt data minimizes the anisotropic information loss due to the missing angular range.

Continued on next page

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Thick (0.25-1.0 μm) sections of cells are imaged and photographed in the HVEM from different viewing directions. The resulting tilt (single axis or double-tilt) series allows one to study, by means of computer manipulations, the structure of subcellular organelles and their three-dimensional relationships. The tilt series of micrographs is scanned using the microdensitometer, and the area of interest is selected interactively on the graphics terminal. The projections are then aligned by cross-correlation[1] and processed with the ART[2] or modified back projection method.[3] A 3-D point spread function is computed using the experimental geometry, to aid with the interpretation of fine structure. The 3-D raster data resulting from the reconstruction are thresholded and presented in solid-object representation.[4]
- *Current Research:* Experience in 3-D reconstruction of nonperiodic objects has been obtained from previous projects including the structure of the 20 nm chromatin fiber[5] and reconstruction of the *Escherichia coli* 30 S ribosomal subunit from random projections.[6] Although the data in both projects were obtained with conventional electron microscopes, the methods of analysis are immediately applicable to thick sections viewed with the HVEM. Pilot reconstruction of a microtubule axoneme, imaged in the HVEM, has been carried out. Our current research emphasis is on the 3-D organization of cytoskeletal components, and the first project conducted with the completed facility will be the analysis of the centriole architecture.
- *New Concept/Capability:* The 3-D reconstruction facility combined with the HVEM tilt/rotation capability provides a means of visualizing the ultrastructure of cellular components. This new facility, when operational, will be able to fully exploit the enhanced penetration power of the HVEM.

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Services Available

- *Service Personnel:* Resource staff members discuss proposals for collaborative projects; coordinate the use of HVEM time with the director of the HVEM facility, Dr. D. F. Parsons; and assist in the preparation of the specimen, designs of the tilt experiment, and processing and representation of data. The facility is currently being developed and will not be available for collaborative projects until October 1, 1985.
- *User Laboratory Space:* No separate user space provided, but work stations will be reserved for selected collaborative projects.
- *User Contact Person:* Joachim Frank, Ph.D. (518) 474-7002.

Research Emphasis or Application

[1]Frank, J., The role of correlation techniques in computer image processing. In: *Computer Processing of Electron Micrographs*. P. W. Hawkes, ed., 1980.
[2]Gordon, R., Bender, R., Herman, G. T., Algebraic reconstruction techniques (ART) for three-dimensional electron microscopy and x-ray photography. *Journal of Theoretical Biology* 29:471, 1970.
[3]Gilbert, P. F. C., The reconstruction of a three-dimensional structure from projections and its application to electron microscopy. *Proceedings of the Royal Society of London* 182:89, 1972.
[4]Radermacher, M., and Fran, J., Representation of three-dimensional reconstructed objects in electron microscopy by surfaces of equal density. *Journal of Microscopy*, In press.
[5]Subirna, J. A., Munoz-Gueza, S., Radermacher, M., and Frank J., Three-dimensional reconstruction of chromatin fibers. *Journal of Biomolecular Structural Dynamics* 1:705, 1983.
[6]Verschoor, A., Frank, J., Radermacher, M., Wagenknecht, T., and Boublik, M., Three-dimensional reconstruction of the 30s ribosomal subunit from randomly oriented particles. *Journal of Molecular Biology*, In press.

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Biological Structure and Function: Electron Microscopy

Title of Resource and Principal Investigator

Resource:
Electron Microscope Facility

Principal Investigator:
Joseph S. Wall, Ph.D.
Department of Biology
Associated Universities, Inc.
Brookhaven National Laboratory
Upton, New York 11973
(516) 282-2912/3372

Services Available

Instruments: High-resolution (2.5 A) scanning transmission electron microscope (STEM), 40 keV; ultrahigh-vacuum evaporation and freeze-drying systems for specimen preparation; optical diffractometer; and image analysis computer.

- *Special Features:* "Vibration-free," liquid nitrogen temperature cold stage and specimen changer; field emission electron source; low-dose, dark-field imaging; single heavy-atom visualization; computer-controlled scanning and data acquisition; molecular weight measuring capability; off-line image analysis using a local computer.
- *Service Personnel:* Facility personnel available to assist in specimen preparation and observation. Staff members available to aid in image interpretation and analysis.
- *User Laboratory Space:* User office and laboratory space provided.
- *User Contact Persons:* Joseph S. Wall, Ph.D., (516) 282-2912/3372; James Hainfeld, Ph.D., (516) 282-3367.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The resource provides high spatial resolution (2.5 A) imaging of biological specimens at low dose and low temperature. Detectors normally used for imaging eliminate phase-contrast artifacts. Well-defined detector geometry and digital recording of images make quantitative analysis of images possible. Molecular weight of isolated particles can be determined from electron scattering to an accuracy of 0.1 percent-10 percent, depending on specimen.[1] Single heavy-atom staining of ordered arrays or staining of single particles with heavy-atom clusters can be used to localize sites of interest. Heavy-atom binding can be studied as a function of dose by using mass measurements.[2] Atom motion and structure of substrates can be imaged.
- *Current Research:* Structure of filamentous and spherical viruses, DNA-protein complexes,[2] and purified proteins (dynein,[3] glutamine synthetase, fibronectin, etc.). Development of new specimen preparation techniques such as improved substrates, improved heavy-atom stains, and frozen-hydrated specimen preparation and imaging. Development of new analysis capabilities: molecular weight measurement, Fourier image analysis, and pattern recognition of atoms and/or molecules.
- *User Research:* Structural studies of nucleosomes, DNA-protein complexes, muscle proteins, rat brain synapses, fibrinogen, ribosomes, apoferritin, collagen fibers, intermediate filaments,[4] coated vesicles, glycoproteins, phycobilisomes, small platinum or mercury clusters as stains, meningopolysaccharides, and chromosomes.
- *New Concept/Capability:* The high-resolution cold stage (−160°C) significantly reduces mass loss and heavy-atom motion. Reduced mass loss is necessary to observe such structures as polysaccharide fibers and myosin tails. Quantitative microscopy makes possible the identification of molecules in an image by molecular weight. Reduced mass loss at low temperature significantly improves the accuracy of these measurements. Mass measurements have proven useful in determining the

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Research Emphasis or Application

number of subunits in an oligomer and in quantitating the binding of heavy atoms to biological molecules and determining the stability as a function of dose. Quantitative microscopy has made possible an experimental study of heavy-atom signal and obscuring substrate noise, giving an empirical formula for predicting in advance the feasibility of a specific labeling experiment.[3] Small improvements in image signal or reductions in substrate noise can be detected, leading to development of better substrates, stains, and imaging conditions. Low-dose, dark-field imaging makes possible the study of unstained freeze-dried biological molecules at 10-20 A useful resolution. This resolution continues to improve as specimen preparation techniques and imaging conditions are refined. Heavy-atom cluster compounds can be used to localize specific sites at about 10 A resolution.

[1]Wall, J., In *Introduction to Analytical Electron Microscopy*. (Hren, J. J., Goldstein, J. I., and Joy, D. C., eds.). New York, N.Y., Plenum Publishing Corporation, 1979, p. 333.
[2]Hough, P. V. C., Mastrangelo, I. A., Wall, J. S., Hainfeld, J. F., Simon, M. N., and Manley, J. L., *Journal of Molecular Biology* 160:375-386, 1982.
[3]Johnson, K. A., and Wall, J. S., *Journal of Cell Biology* 96:669-678, 1983.
[4]Steven, A. C., Wall, J., Hainfeld, J., and Steinert, P. M., *Proceedings of the National Academy of Sciences* 79:3001-3015, 1982.

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Biological Structure and Function: Electron Microscopy

Title of Resource and Principal Investigator

Resource:
Electron Microscope Facility for Biomedical Research

Principal Investigator:
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University of Wisconsin
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Coinvestigator:
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University of Wisconsin
167 Animal Science Building
1675 Observatory Drive
Madison, Wisconsin 53706
(608) 263-3147

Services Available

- *Instruments:* AEI EM-7, 100- to 1,000-keV transmission electron microscope. Measured resolution, 0.14 nm lattice 0.3 nm, point to point; diffusion and ion-pumped vacuum evaporators; Polaron LHe[1] Slammer's sample freezer; freeze dryer; laser optical diffractometer; low voltage SEM. Facilities for computer image processing of low-dose images.
- *Special Features:* Axis-centered ±45° and ±60° tilt stages and rotation-free imaging system facilitate making of stereo pairs; newly designed high-resolution image intensifier television viewing system with digital frame store interfaced to PDP-11/23 computer with array processor; high-resolution LN_2 cold stage; hydration stage; equipment for analyzing stereomicrographs and making stereoslides; specimen chamber vacuum 2×10^{-7} torr; microscope magnification range, 63-1,000,000 X; full electron diffraction capabilities; simple operation and rapid, side-entry sample exchange; rotary hollow-cone dark-field illumination system; minimal exposure system; push-button selection of up to 5 preset magnification/illumination conditions; and digitally operated focus control.

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource provides the instrumentation necessary to provide high-resolution stereoimages of samples 10-50 times thicker than those that can be viewed in the normal 100 keV transmission electron microscope. Ultrastructural morphology can be supplemented by techniques to localize specific proteins within the cell, using immunocytochemistry or autoradiography carried out on these thicker sections. Entire tissue culture cells or isolated subcellular structures such as chromosomes can be viewed at high resolution, often without the need for staining. These abilities greatly simplify studies of the three-dimensional architecture of cells and tissues. HVEM holds the potential for higher spatial resolution than is available with normal EM, and we are actively involved in realizing this potential and applying it to the study of crystalline biological macromolecules. Finally, the relatively large amount of free space present around the specimen area (the objective lens gap) simplifies the operation and construction of controlled-atmosphere and low-temperature stages, which permit the observation of samples that have not been exposed to normal preparative procedures. These capabilities have been applied to a wide variety of ultrastructural problems, one of the most important of which is the elucidation of the substructure of the chromosome.
- *Current Research:* Chromosome structure;[1] kinetochore structure and function;[2,3] low-dose, high-resolution study of microcrystals of biological macromolecules;[4] structure of the synaptic region of isolated nerve fibers; structure of growth cones of neurons grown in culture; cell wall synthesis in filamentous fungi; structural basis of tissue cell motility; structure of coarse and fine fibrin films; structure of insect eye and nervous tissue; simultaneous visualization of surface structure and internal cell organelles;[5] preservation of cytoskeleton in cell whole mounts by critical point drying[6] and freeze drying; structure of whole-mount platelets during activation and localization of surface markers using colloidal gold immunocytochemistry; immunolabeling of cytoskeletal proteins by microinjection of single cells.

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Biological Structure and Function: Electron Microscopy

Services Available

- *Under Development:* Program of high-resolution studies on frozen biomacromolecular crystals. Video-enhanced light microscopy of low-voltage scanning electron microscope.
- *Service Personnel:* Three staff members are available to assist users with specimen preparation, instrument use, and evaluation of results. They also develop all micrographs, maintain and improve equipment, and are available to collaborate or advise. Microscope in operation 60 hours/week.
- *User Laboratory Space:* Office and specimen preparation facilities available to out-of-town users.
- *User Contact Person:* James Pawley, Ph.D., (608) 263-3147/6288

Research Emphasis or Application

- *New Concept/Capability:*

—*Chromatin Folding:* Cell proliferation requires the transient packing of DNA-protein fibers into highly condensed metaphase chromosomes prior to division. Perhaps the most important level of fiber folding in metaphase chromosomes is the formation of radial loops, each including some 10-30 nm of DNA and each anchored into some core structure. The critical contribution from this laboratory to the radial loop model has been the development of evidence supporting the hypothesis that the core structure is not an independent organelle (scaffold) but that it consists of specialized regions of unusual protein composition within the continuous chromatin fibers themselves. These regions become highly compacted during metaphase condensation, forcing intervening regions into loop configurations.

- *Kinetochore Structure:* The actual process of cell division involves the movement of chromosomes to the two daughter cells. The movement is mediated by microtubules attached to an elaborate structure, the kinetochore, which is formed at specialized chromosome regions (centromeres). Using stereomicroscopy of thick sections, we have shown that the outer kinetochore disk in mammals consists of regularly arranged loops of chromatin fibers to which microtubules attach. Thus, the kinetochore may be conceptualized as a special example of regional differentiation of the unit chromatin fiber. This same approach is now used to analyze the "ball-and-cup" kinetochore in insects and higher plants.

[1]Ris, H., and Korenberg, J., In *Cell Biology* (Prescott and Goldstein, eds.). New York, N.Y., Academic Press, vol. 2, 1979, pp. 268-361.
[2]Witt, P. L., Ris, H., and Borisy, G. G., *Chromosoma* 81:483-505, 1980.
[3]Ris, H., and Witt, P. L., *Chromosoma* 82:153-170, 1981.
[4]Pawley, J. B., *Ultramicroscopy* 13:387-407, 1984.
[5]Ris, H., *37th Annual Proceedings of the Electron Microscopy Society of America*, p. 166, 1979.
[6]Ris, H., *38th Annual Proceedings of the Electron Microscopy Society of America*, p. 812, 1980.

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Biological Structure and Function: Electron Microscopy

Title of Resource and Principal Investigator

Resource:
High-Voltage Electron Microscope Biological Facility for the East

Principal Investigator:
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Wadsworth Center for Laboratories and Research
New York State Department of Health
Empire State Plaza
Albany, New York 12201
(518) 474-7047/7049

Coinvestigators:
William F. Tivol, Ph.D.
Physicist-in-Charge of the HVEM
(518) 474-6346/3364

Conly L. Rieder, Ph.D.
Cell Biologist
(518) 474-2560

Services Available

• *Instruments:* AEI EM7 Mk II. 100- to 1,200-kV transmission electron microscope, 4 A resolution; JEOL 200. 50-200 kV transmission electron microscope, 5 A resolution, for screening thick sections and some environmental chamber work.

• *Special Features:*
AEI EM7: Stereoscopy of thick sections and whole-cell mounts; vibration-free double-tilt stage (side entry) ±57°, ±39°; setting of effective tilt and azimuth assisted by programmed calculator; a single-tilt stage (±30°) with 360° rotation and axis-centered tilt stage is available, as is a cold stage; a side-entry environmental chamber is under construction; Hilger and Watts Stereo Viewer; *computerized tomography* of multi-tilted single thick sections being developed; *high-resolution capability:* 4 A resolution with detached specimen tip or double-tilt stage; reduced contamination of specimen due to use of large ion pumps on both column and accelerator; liquid nitrogen traps and anticontamination device cooled by either liquid helium or liquid nitrogen; double-tilt cold stage; liquid nitrogen cooled to −150°C; tilt ranges ±39°, ±60°. *Imaging modes:* 630X to 1,000,000X, with zero image rotation 2,500-800,000X; special low-magnification mode for panoramic views, 63-630X; wobbler for low-magnification focusing; high-resolution dark-field (central beam-stop strioscopy, electronic rotating hollow-cone illumination under development); *electron diffraction* (ED): selected area to 0.35 micrometer diameter and camera length

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Research Emphasis or Application

Data Achievable with Resource's Methodologies: Entire cell contents can be viewed at one time in a critical-point, dried whole-cell mount or examined with a small number of semithick (0.25-0.50 micrometer) or thick (1.0-5.0 micrometer) sections. The semithick sections can be screened readily by light microscopy before electron microscopy. Stereophotographs can be easily and rapidly obtained by tilting the thick section at angles and examining the stereo-pair in a viewer. Serial thick sections can be profiled and the profiles reconstructed by computer graphics to summarize the main features of the complete set of sections. Three-dimensional viewing clarifies shape, connections, and terminations of structures, providing insight into their function. The complete cellular distribution of cytochemical reaction products also can be obtained with three-dimensional viewing. A dye-stained thick section surveyed by light microscopy subsequently can be stained by uranyl and lead to provide either low magnification panoramas or high magnification detail in the HVEM on the identical section, allowing identification of cell types. Thick specimens also provide the same brief exposure autoradiography obtainable by light microscopy but allow resolution of small structures. The HVEM provides low-magnification panoramic views of thick specimens.

• *Current Research:* Importance of the contractile-migration apparatus of invasive cells as demonstrated by stereophotos and computer graphics reconstructions of tumor cells passing through spaces of less than 1 micrometer diameter[4] in the elastic reticulum. Low magnification panoramas of thick sections of epidermis were used to quantitate the appearance of ribosome-rich "dark cells" after promoter and carcinogen treatment.[4] ^{14}C thick-section HVEM autoradiography is being optimized to provide quantitation of invasion by ^{14}C-labeled carcinoma cells. The cytoskeleton of migrating tumor cells is being studied in micropore filters for effects of chemotaxis and chemokinesis. The mitotic spindle apparatus is being compared in many organisms[3] with special emphasis on kinetochore microtubule formation, the centrosome cycle, and cilia. Portions of the mitotic spindle are being reconstructed in three dimensions by computerized

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Services Available

50-500 cm; small-angle ED-camera length 50-500 m; *environmental chambers:* differentially pumped environmental chamber (DIFPEC); inner apertures 100 micrometers, outer apertures 200 micrometers; gas pressures up to 1 atmosphere; full 5 A resolution without gas and only slight reduction by the equilibrium vapor pressure of water vapor; NPL side-entry, Alundum-window type of environmental chamber; *image recording* cut-film camera holds up to 50 6.5 x 9.0 cm films (Kodak 4489); temperature-controlled, nitrogen-burst agitated developing tank, sensitive x-ray film used for electron diffraction and imaging; television camera under development. *X-ray microprobe* element analysis at high voltage; specially designed and shielded Kevex energy dispersive x-ray detector with Tracor Northern TN 2000 multichannel analyzer and data reduction software. *Electron energy loss spectroscopy (EELS) microprobe element analysis* at high voltage; suited for light elements at low concentration; under development.

- *Service Personnel and Services:* Operators set up the microscope and assist user in inserting specimens, loading and unloading camera and developing films, and operating and aligning the microscope. Microscope up-time is better than 85 percent. A computerized fault-trace program with some automatic logging of essential on-line parameters is under develop-

Continued on next page

Research Emphasis or Application

tomography of multi-tilted thick sections. The pore subunits of the outer membrane of mitochondria are being studied by HVEM dark-field[4] and image processing. Cocultured pinealocytes and neurons show new synapse features. Three-dimensional analysis is being applied to crystalline arrays in pathological muscle cells and to membrane arrays in xenobiotic-exposed guinea pig liver. To analyze elements at high voltages, an energy dispersive x-ray microprobe analysis system is used, and an electron energy loss spectroscopy (EELS) system is being developed. Both systems will be used to examine thick objects. Elements of low atomic number will be examined with EELS, and elements of higher atomic number will be examined with x-ray microprobe. Outside users have studied a new species of *Blastoschizomyces;* three-dimensional reconstruction of the thylakoid membranes of *Agmenellum quadruplication;* vesicular structure in endothelial cells; leakage pores of phototoxic rat retina vessels; HVEM autoradiography of ^{35}S-sulfate incorporation into the extracellular matrix of the red alga chondrus; the cytoskeleton of *Allogromia sp.* exhibiting external motility of attached particles using correlated light microscopy of living cells and HVEM; and possible tonofilament nucleating centers of epithelial cells.

- *New Concept/Capability:* The ultrashort wavelength electron radiation of the HVEM offers new biological ultrastructural analysis capabilities. Reduced chromatic aberration enables high-resolution dark-field imaging of unstained biological structures. Bright-field HVEM has a theoretical resolution better than 2 A point to point. The ultrashort wavelength promises increased theoretical sensitivity for both x-ray and EELS microprobe element analysis, especially for thicker objects. Extension of the three-dimensional capability, both through simple stereoscopy and computerized tomography, will be used to study function. Computerized tomography can unscramble superimposed projected structures in very thick specimens (up to 5 micrometers) where membranes are still resolved. Full penetrating power can be utilized without special selective stains. Studies using the environmental chamber have demonstrated that the diffraction

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Research Emphasis or Application

[1]Chait, B. T., Shpungin, J., and Field, F. H., Fission fragment ionization mass spectrometry of polyethers. *International Journal of Mass Spectrometry and Ion Physics* 58:121, 1984.
[2]Chait, B. T., and Field, F. H., ^{252}Cf fission fragment ionization mass spectrometry of chlorophyll a. *Journal of the American Chemical Society* 106:1931, 1984.
[3]Kreek, M. J., Bencsath, F. A., Fanizza, A., and Field, F. H., Effects of liver disease on fecal excretion of methadone and its unconjugated metabolites in maintenance patients. *Biomedical Mass Spectrometry* 10:544, 1983.
[4]Bencsath, F. A., Bradlow, H. L., and Field, F. H., Determination of total body water by deuterium isotope dilution methods using chemical ionization mass spectrometry. *Biomedical Mass Spectrometry* 10:596, 1983.
[5]Field, F. H., Fast atom bombardment study of glycerol: Mass spectra and radiation chemistry. *Journal of Physical Chemistry* 86:5115, 1982.

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Services Available

ment. Specimen preparation assistance is available for new users who need it. Mailed-in specimens are examined prior to visit and sample stereo micrographs of selected features are taken. Two-shift operation from 8:30 a.m. to 4 p.m. and 4 to 11:30 p.m., 5 days a week.

- *User Laboratory Space:* Desk and specimen preparation space available. Special laboratory is available for visiting scientists. Includes tissue culture, biochemical, sectioning, grid-coating light microscopy with video recording, micrograph digitizing (Perkin-Elmer flat-bed densitometer) and computer facility.
- *User Contact Persons:* Dr. Donald F. Parsons, (518) 474-7047/7049; Dr. Lee D. Peachey, (215) 243-5788; contact Dr. William Tivol at (518) 474-6646 or 473-3384 to schedule time on the HVEM.

Research Emphasis or Application

Intensity data of electron diffraction patterns of minute, wet, unfixed, and unstained protein crystals warrant full crystallographic analysis to obtain high-resolution imaging of protein molecules. EELS allows elemental analyses of whole wet cells in the environmental chamber. Combination of microbeam scanning with energy filtration by EELS promises improved contrast and resolution of images of wet cells in the environmental chamber.

[1]King, M. V., Parsons, D. F., Turner, J. N., Chang, B. B., and Ratkowski, A. J., *Cell Biophysics* 2:1, 1980.
[2]Turner, J. N. (ed.), *Methods in Cell Biology* V. 22. Academic Press, 1981.
[3]Rieder, C. L., *International Review of Cytology* 79:1, 1982.
[4]Mannella, C. A., and Ratkowski, A. J., *Ultramicroscopy* 11:24-31, 1983.

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Biological Structure and Function: Electron Microscopy

Title of Resource and Principal Investigator

Resource:
High-Voltage Electron Microscopy of Biological Systems

Principal Investigator:
J. Richard McIntosh, Ph.D.
Molecular, Cellular, and Developmental Biology
University of Colorado
Boulder, Colorado 80309
(303) 492-8533

Coinvestigators:
Mircea Fotino, Ph.D.
(303) 492-8819
John Kinnamon, Ph.D.
(303) 492-8746

Services Available

- *Instruments:* 1-MeV electron microscope, JEOL model JEM 1000.
- *Special Features:* Usable voltage range, 500 kV to 1 MV; six-specimen carousal, grid diameter of 3 mm; magnification range, 150-250,000 X; resolution better than 3 A lattice; routine goniostage tilting range ± 30° about any axis, and ± 60° with special holder; dark field by tilted beam and stryoscopy aperture; electron diffraction camera lengths 1-4 m, film size 3¼ x 4 inches; cryostage cooled by liquid nitrogen operational for viewing specimens from 78°K to room temperature; capabilities for taking, viewing, and assembling stereomicrographs.
- *Under Development:* Improved cryostage for high-performance imaging down to liquid helium temperatures; hybrid scanning system for direct electronic imaging; spectrometer for energy filtering and analytical applications; computer facilities for 3-D reconstruction from serial sections and image processing.
- *Service Personnel:* Resource personnel operate the microscope for users during learning phase. Support equipment available. Instrument in operation 40 hours/week. Mail-order service for preliminary or followup inspection of specimens.
- *User Laboratory Space:* Separate user space provided for two visiting investigators.
- *User Contact Person:* Mircea Fotino, Ph.D., (303) 492-8819.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The high-voltage electron microscope offers the biological investigator a unique possibility of viewing the organization of fine structures in preparations whose thickness defies imaging by other forms of microscopy. These preparations include whole cultured cells, isolated entities such as chromosomes, and thick tissue sections. Structurally rich images of good resolution can be obtained with specimens several micrometers thick, especially when they have been selectively stained. The increased specimen thickness and the penetration capability thus add a third dimension to visualization of fine-structure organization. Stereoimages are taken routinely to facilitate the spatial viewing and reconstruction of intracellular systems. The recently added capability of imaging at low temperatures allows the investigation of hydrated (frozen) specimens and ultrastructural observations by fast freezing and etching in situ as an alternative for conventional chemical fixation.
- *Current Research:* Structure of the isolated mitotic apparatus; 3-D structure of mammalian taste buds and of taste bud development; neuromuscular functions; aging features in whole WI-38 cells; three-dimensional structure of the cytoplasmic matrix; pattern recognition in disposition of microtubule organizing centers in plant and animal cells; three-dimensional antigen localization at high resolutions; origin of motive force in pigment translocation in chromatophores; morphogenesis of myofibrils; structure of polytene chromosomes; mechanisms for cell motility; imaging of cytoskeleton after detergent extractions; resolution in thick sections; microscopy of frozen-hydrated specimens; and direct electronic imaging for analytical applications and/or computer processing.
- *New Concept/Capability:* Most of the investigators who have had access to this installation have found that the evidence contained in their HVEM micrographs is of broader significance than anticipated, thus leading to new perspectives on their subject matter. In general terms, it may be said that most concepts of the cell have been better defined by improved resolutions of spatial organization in all dimensions. In Keith Porter's laboratory, the microscope has been used extensively to study the three-dimensional structure of the cytoplasmic matrix. Out of these investigations have developed

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Research Emphasis or Application

entirely new concepts of the structure and organization of this part of the cell. Instead of being a cytosol, it is a three-dimensional lattice (gel) of slender trabeculae (5-10 nm in diameter). This structure contains and positions, in nonrandom distribution, the formed components of the cytoplasm, such as ribosomes and microtubules. It appears to be organized radially around the cell's central apparatus to constitute a large, complex, and polymerized unit, the cytoplast. Also emerging from the core research currently in progress is a novel capability for the direct electronic manipulation of TEM images.

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Biological Structure and Function: Electron Probe

Title of Resource and Principal Investigator

Resource:
National Electron Probe Resource for Analysis of Cells

Principal Investigator:
Claude Lechene, M.D.
Harvard Medical School
NBREM Laboratory
221 Longwood Avenue
Boston, Massachusetts 02115
(617) 732-2063

Services Available

- *Instruments:* One electron probe microanalyzer Cameca MS46, with 4-wavelength dispersive x-ray spectrometers. One electron probe Cameca MBX with three wavelength-dispersive and one energy-dispersive spectrometers (Tracor Northern TN2000, Kevex detector).
- *Special Features:* MS46 automated stage with 4x6 cm displacement ± 1 μM reproducibility (to be installed). MBX: 10^{-8} Torr vacuum oil free. Cold stage by liquid nitrogen circulation. Extensive software data reduction.
- *Service Personnel:* Laboratory staff are available on a limited basis for training in cell preparation, analysis, and data reduction.
- *User Laboratory Space:* Available for training in aspects of cell culture related to electron-probe analysis.
- *User Contact Person:* Claude Lechene, M.D., (617) 732-2063.

Research Emphasis or Application

- *Current Research:* Relation among cell volume, ionic fluxes, and intracellular pH; relation among stability of cell volume, volume regulation, cytoskeleton, and cytoplasmic gel; ionic pathways in fibroblasts and in early primary culture of epithelial renal proximal cells; development of specific transport properties in renal epithelial cells; metabolic cooperation (MDCK cell line); expression of functional polarity in epithelial cells; ionic shifts during the cell cycle (fibroblasts, primary culture of epithelial renal proximal tubular cells); analysis of extremely small number of cells allows the resource to study early primary cell culture before dedifferentiation; analysis of multiple elements within the same cells, in particular Na^+, K^+, and Cl^-, allows direct study of stoichiometry of net ionic fluxes; electron probe autoradiography allows comparison of ionic content with metabolic events; analysis of population of cells one by one uncovers the possibility of cellular functional heterogeneity.
- *Data Achievable with Resource's Methodologies:* Measurements of chemical elements (from carbon up in the periodic table) in small population of single cells in culture, analyzed individually; measurements of kinetics of ionic fluxes with approximately 20 cells per time point. (Effective permeabilities, Na-K − ATPase coupling ratios); histogram of chemical elements' distribution in a cell population; electron probe autoradiography (3H thymidine, α-methylglucoside) relating ionic to cell cycle or metabolic events; estimate of cell volume, from the sum of $Na^+ + K^+ + Cl^-$; estimate of intracellular fixed charges, from electroneutrality principle; analysis of frozen hydrated cells (in development).

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Biological Structure and Function: Electron Spectroscopy

Title of Resource and Principal Investigator

Resource:
National Electron Spectroscopy for Chemical Analysis (ESCA) and Surface Analysis Center for Biomedical Problems (NESCA/BIO)

Principal investigator:
B. D. Ratner, Ph.D.
Department of Chemical Engineering
BF-10
University of Washington
Seattle, Washington 98195
(206) 545-1005

Coinvestigators:
Allan S. Hoffman
(206) 543-9423
Thomas A. Horbett
(206) 545-1392

Services Available

- *Instruments:* Surface Science Laboratories SSX-100 ESCA.
- *Special Features:* Spatial resolution in a 150 mμ circle; high-speed high-resolution computerized sample handling; enhanced computerized data reduction and storage capability; ability to study the surfaces of frozen, hydrated specimens.
- *Service Personnel:* Brien J. McElroy, ESCA laboratory coordinator; Mady Lund, executive secretary.
- *User Laboratory Space:* 500 square feet equipped with clean-room areas, a wet-process station for sample cleaning and preparation, microscopes, sample drying and storage facilities, a fume hood, and general laboratory preparation areas. Special arrangements for tissue culture and protein adsorption studies are possible.
- *User Contact Person:* Mady Lund, (206) 545-1229.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Electron spectroscopy for chemical analysis (ESCA) utilizes the photoelectron effect to provide information needed to analyze the surface chemistry and structure of a specimen. The specimen, contained in an ultrahigh-vacuum environment, is exposed to a beam of low-energy X rays. The number and energy of photoelectrons emitted from this excitation is determined. From the spectrum obtained, the following information can be extracted about the uppermost 10-100 A of a specimen:

—All elements present in the surface region (except H and He);

—Semiquantitative analysis of those elements found in the surface;

—Molecular binding environment and oxidation state for most atoms in a surface;

—Differentiate aromatic and aliphatic structures (in some cases);

—Suggestions concerning the nature of bonding interactions at interfaces;

—Surface electrical properties and surface charging;

—Nondestructive depth profiling (composition as a function of depth in the surface);

—Destructive depth profiling of inorganic materials using ion etching or RF plasma methods;

—Identification of specific organic functional groups based upon binding-energy shifts and chemical derivatization studies; and

—Fingerprint identification of materials by using valence band or molecular-orbital spectra.

- *Current Research:* The research goals for NESCA/BIO are directed toward understanding the nature of interactions that occur at biological interfaces such as these: tissue-body fluid; synthetic-material blood; tooth saliva; etc. The majority of processes that occur in living organisms occur at interfaces, and the ability of ESCA to measure in situ the minute amounts of materials involved makes it especially attractive for such studies.
- *New Concept/Capability:* Most ESCA instruments have been designed for surface physics studies. Such studies have special requirements for sample handling and cleanliness that do not pertain to the needs of the biochemical research community. The ESCA instrument at NESCA/BIO has been especially configured to

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Research Emphasis or Application

make it highly usable for organic materials in a hydrated or dehydrated form and to be capable of handling large numbers of users.

[1]Hanson, S. R., Harker, L. A., Ratner, B. D., and Hoffman, A. S. Evaluation of artificial surfaces using baboon arteriovenous shunt model. Biomaterials 1980, Advances in Biomaterials, Vol. 3, G. D. Winter, D. F. Gibbons, and H. Plenk, Jr., eds., John Wiley and Sons, Chichester, England, 1982, pp. 519-530.

[2]Paynter, R. W., Ratner, B. D., Horbett, T. A., and Thomas H. R., XPS studies on the organization of adsorbed protein films on fluoropolymers. *Journal of Collagen Interface Science* 101:233-245, 1984.

[3]Ratner, B. D., Surface characterization of materials for blood contact applications. Biomaterials: Interfacial Phenomena and Applications, ACS Advances in Chemistry Series, Vol. 199, S. L. Cooper and N. A. Peppas, eds., American Chemical Society, Washington, D.C., 1982, pp. 9-23.

[4]Ratner, B. D., Analysis of surface contaminants on intraocular lenses. *Archives of Ophthalmology* 101:1434-1438, 1983.

[5]Ratner, B. D., Surface characterization of biomaterials by electron spectroscopy for chemical analysis. *Annals of Biomedicine in England* 11:313-336, 1983.

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Biological Structure and Function: Electron Spin Resonance

Title of Resource and Principal Investigator

Resource:
National Biomedical Electron Spin Resonance (ESR) Center

Principal Investigator:
James S. Hyde, Ph.D.
Radiation Biophysics Section
Department of Radiology
The Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, Wisconsin 53226
(414) 257-8570

Services Available

- *Instruments:* ENDOR (Varian E-109 EPR spectrometer with E-1700 ENDOR accessory); ELDOR (Varian E-9 EPR spectrometer with E-800 frequency swept ELDOR accessory); multifrequency ESR (Varian E-9 with 1-2 GHz, 2-4 GHz, 9 GHz, and 35 GHz bridges); time domain ESR (saturation recovery measurements of relaxation times); IBM Instruments, Inc., ER 200D ESR Varian E-4 ESR spectrometer system; IBM Instruments, Inc., ER 200D ESR spectrometer system. Magnetometers and frequency counters available for precise field and frequency measurements (including 35 GHz).
- *Special Features:* Extensive cryogenic equipment including liquid-helium apparatus for all available microwave frequencies; online minicomputer for data acquisition and spectral simulation; excimer laser (Lambda Physics, EMG 101); vacuum line; photochemical facilities including Xe and Hg lamps and monochromator; high-power sonication; stopped-flow ESR apparatus; Perkin-Elmer 320 UV-VIS spectrophotometer; Sorvall RC5B super speed refrigerated centrifuge; Beckman Airfuge ultracentrifuge; Cahn balance (microgram sensitivity); and other normal chemical and biological laboratory equipment. Microwave test equipment including Hewlett-Packard sweep generator to 40 GHz (8690) and a spectrum-analyzer (8566). Extensive electronic test equipment.

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The ESR center staff has broad experience in use of nitroxide radical spin labels to measure translational and rotational diffusion in biological systems, with particular emphasis on measurement of rotational correlation times in the range of 10^{-3} to 10^{-7} sec and on measurement of slow translational diffusion constants (10^{-6} to 10^{-9} cm^2/sec). These are characteristic values for components of biological membranes. Several spin-probe methods have been developed and are available for measuring oxygen concentrations and diffusion in cells and other complex systems. Identification and characterization of transient and stable free radicals are accomplished in various ways, using the several types of ESR equipment available in the center, including multifrequency ESR and ENDOR. It is often possible to identify paramagnetic metal ions and determine the symmetry of the crystal field, the charge, and the ligation. $^{63}Cu^{2+}$ is being used as an extrinsic probe of the structure of metal-binding sites, primarily using the 2-4 GHz bridge. Study of relaxation processes by means of which paramagnetic centers are maintained in thermal equilibrium with their environment is possible, using the recently developed pulse ESR spectrometer.
- *Current Research:* Physical studies of nitroxide radical spin labels; measurement of spin-label T_1's; diffusion processes in synthetic and biological membranes; measurement of oxygen concentrations in synthetic membranes and of oxygen uptake in cellular systems; spin-label studies of fibronectin; physical studies of melanin; clinical and diagnostic studies of melanin-containing tissues; ESR characterization of immobilized free radicals; phototoxicity in pigmented tissues; oxidation mechanisms and toxicity of catecholamines; spin-trapping and spin-stabilization of oxygen-centered free radicals; copper ligation and charge in copper proteins and copper-containing chemotherapeutic and radiosensitizing drugs; and characterization of higher order effects occurring in transition metal spectra at low microwave frequencies.

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Biological Structure and Function: Electron Spin Resonance

Services Available

- *Under Development:* Microwave bridges from 0.5 to 1 GHz and from 4 to 8 GHz. Separate ENDOR spectrometer at S and L bands (1 to 4 GHz). Pulse ELDOR at X-band.
- *Service Personnel:* Eight ESR spectroscopists available for collaborative research, system instruction, and discussion. Electronics shop, machine shop, and tissue culture laboratory provide supporting services.
- *User Laboratory Space:* User desk space available for extended visits. Facility occupies 8,000 square feet; six separate spectrometer stations.
- *User Contact Persons:* Christopher C. Felix, Ph.D., (414) 257-8760; James S. Hyde, Ph.D., (414) 257-8570.

Research Emphasis or Application

- *New Concept/Capability:* 1-2 and 2-4 GHz ESR spectrometers have been designed and constructed, and are particularly appropriate for the analysis of transition metal systems. Improved spectral resolution from a wide variety of copper-containing systems can be achieved; for example, observation of resolved structure from the "EPR-visible" copper site of cytochrome c oxidase. A new resonator yields higher sensitivity by two to three orders of magnitude in the 1-4 GHz range. At X-band, very small resonators that require only 1 micro ESR of sample have been developed. A spin-probe technique called spin-label oximetry, which requires as few as 1,000 cells, has been used to measure oxygen uptake by cells. Spin stabilization uses diamagnetic metals to stabilize orthosemiquinone-free radicals occurring in biochemical reactions.

[1]Subczynski, W. K., and Hyde, J. S., Diffusion of oxygen in water and hydrocarbons using an electron spin resonance spin-label technique. *Biophysical Journal* 45:743-748, 1984.

[2]Felix, J. B., Popp, C. A., Venkataramu, S. D., Beth, A. H., Park, J. H., and Hyde, J. S., An electron-electron double resonance study of interactions between ^{14}N and ^{15}N steric acid spin-label pairs: Lateral diffusion and vertical fluctuations in dimyristoylphosphatidylcholine. *Biochemistry* 23:2293-2299, 1984.

[3]Lai, C. S., Tooney, N. M., and Ankel, E. G., Spin-label studies of sulfhydryl environment in plasma fibronectin. *FEBS Letters* 173:283-286, 1984.

[4]Kalyanaraman, B., Felix, C. C., and Sealy, R. C., Electron spin, resonance-spin stabilization of semiquinones produced during oxidation of epinephrine and its analogues. *Journal of Biological Chemistry* 259:354-358, 1984.

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Biological Structure and Function: Infrared Spectroscopy

Title of Resource and Principal Investigator

Resource:
National Center for Biomedical Infrared Spectroscopy

Principal Investigator:
Robert I. Leininger, Ph.D., Director
Battelle's Columbus Laboratories
505 King Avenue
Columbus, Ohio 43201
(614) 424-7138

Coinvestigators:
Robert J. Jakobsen
Associate Director
(614) 424-5617
R. Michael Gendreau, M.D.
Facility Manager
(614) 424-6317
David J. Fink, Ph.D.
Coordinator, Collaborative Research
(614) 424-7619

Services Available

• *Instruments:* Spectrometers: Digilab FTS-10 (2); Digilab FTS-20 (1); Mattson Sirius (1); Digilab FTS-15/90 for biological spectroscopy (1). Mattson Starlab data system for spectral manipulation (2). Optics: Temperature-controlled transmission and attenuated total reflection (ATR) cells; standard transmission cells; ATR cell designed for flowing liquids; 6X and 4X beam condensers; diffuse reflectance accessory; reflectance/transmittance microsampling accessory; variable reflection (3, 5, 7, 9) ATR liquid cells.

• *Special Features:* Spectra can be obtained by transmission, diffuse reflection, and attenuated total reflection (ATR). Flow cells are available for kinetic studies of reactions or of adsorption on various surfaces. Two Starlab computers with appropriate software are available for data processing. Spectrometers, computers, consoles, and plotters are on a local area network for data transfer, processing, and display. A newsletter is published several times per year, and a symposium on biomedical spectroscopy is held annually in conjunction with the Electron Spectroscopy Chemical Analysis Resource at the University of Washington. For further information about the newsletter or symposium, contact Ms. Susan M. Toth at (614) 424-4821. Collaborative research will be done with outside investigations in which infrared spectroscopy can make a contribution. For further information on collaborative programs, contact Dr. David J. Fink at (614) 424-7619.

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Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* Spectra of solid, liquid, and dissolved samples (aqueous or nonaqueous solvents) can be obtained in transmission, reflection, ATR, or microsampling modes. Spectral frequencies of 10 to 7800 cm^{-1} can be used at resolutions of 0.2 cm^{-1}. Scanning rates are as fast as 0.1 scans/sec so that kinetic studies can be made. Data reduction software include such operations as deconvolution and derivatization.

• *Current Research:* Kinetic studies of the adsorption of proteins from aqueous media; quantitative and qualitative identification of proteins from complex mixtures using both transmissions and ATR spectra; identification of conformational changes in proteins; determinations of the structure of ribonuclease A; antibody-antigen reactions; effect of water on protein structure; collagen fibrillogenesis; lipid and membrane structure; application of FT-IR to process control in fermentation; development of software to improve and standardize spectral data manipulations such as baseline straightening and water subtractions. Collaborative research projects with outside investigators are sought.

• *New Concept/Capability:* FT-IR instruments and flow cells specially designed for ATR experiments give unique capabilities for the study of both adsorbing and adsorbed proteins on a variety of surfaces. This, along with transmission studies in which the protein environment (pH, temperature, solvent, concentration, etc.) is varied, has extended the spectral interpretation of protein structure and behavior. Microscopic IR techniques permit extension of IR studies to biological specimens available only in very limited quantities. A beginning has been made in the use of FT-IR for monitoring industrial biotechnology processes. For example, in a fermentation, nutrient and product levels have been measured in virtually real time by FT-IR. Because spectral interpretation, qualitative or quantitative, especially of mixtures,

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Services Available

- *Service Personnel:* Center staff is available for assistance in operation of the instruments and in the treatment and interpretations of the spectra. Medium- and long-term users are expected to provide operators. Instruction is available at the Center and through short courses that can be held off site. Postdoctoral appointments are made and graduate students accepted for training. Spectra and their interpretation can be made by Center staff by arrangements through the Service Coordinator. The Center supplies materials related to the instruments and the user supplies materials related to the samples.
- *User Laboratory Space:* Instrument time and sample preparation facilities are available during normal working hours and by arrangement during evenings and weekends for longer term visitors.
- *User Contact Persons:* R. Michael Gendreau, M.D., Center Visitors, (614) 424-6317; Mr. Robert J. Jakobsen, Short Courses, (614) 424-5617; Ms. Katrina B. Smith, Service Work, (614) 424-5160.

Research Emphasis or Application

requires such high levels of skill and training, an effort is planned to investigate computer-aided spectral analysis. It is expected that this effort will benefit greatly from the recent advances by the Syracuse NMR Resource. A parallel effort on the compiling of reference spectra of biologicals of interest is also planned. In this, spectra of compounds in the pure state, as solutes, and as appropriate for use as adsorbates, will be obtained. These will be available to the research community. A portion of this work will be done in conjunction with the ESCA Resource at the University of Washington so as to make the structural information complete.

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Title of Resource and Principal Investigator

Resource:
San Francisco Laser Center

Principal Investigator:
Ignacio Tinoco, Jr., Ph.D.
Chemistry Department
University of California
Berkeley, California 94720
(415) 642-3038

Coinvestigator:
Harden M. McConnell, Ph.D.
Chemistry Department
Stanford University
(415) 497-4571

Services Available

- *Instruments:* Pulsed lasers: Q-switched Nd:YAG, Excimer, pulsed dye, Flashlamp pumped dye. Combined tunability from 217 nm to >1000 nm. CW lasers: argon ion, krypton ion, single-frequency dye, color-center, CO_2. Plus a full range of diagnostic accessories for use with these lasers. Detection electronics include box-car integrators, lock-in amplifiers, photomultiplier, and a photon-counter.
- *Special Features:* The Center operates on a "lending library" concept in which lasers are available for short-term loan to individuals interested in using them in their scientific research. Emphasis is on feasibility studies, innovative but high-risk experiments, and measurements involving short-term use of one or more lasers.
- *Service Personnel:* The staff of the San Francisco Laser Center provides instructions on the proper use of the laser and general consultation on various aspects of lasers and their performance.
- *User Laboratory Space:* The lasers are delivered to and installed in the user's laboratory. This facility is not prepared to support onsite experiments.
- *User Contact Person:* Andrew H. Kung, Ph.D., (415) 642-8506.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Experiments involving laser-induced fluorescence, resonance Raman, absorption spectroscopy, nonlinear spectroscopy, differential scattering, and photobleaching are possible. Processes involving a timescale from nanoseconds to CW can be studied.
- *Current Research:* Development of instrumentation for the study of circular intensity differential scattering of chiral macromolecules; molecular motion of molecules involved in cell-cell recognition; wavelength dependency of photochemical formation of previtamin D_3; laser-CIDNP studies of protein structure and dynamics.
- *New Concept/Capability:* This resource provides the unique opportunity of using different types of lasers to test concepts in the user's own laboratory. New instrumentation such as the CIDS apparatus, hard UV ion lasers, and a tunable VUV source are being developed for biomedical applications.

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Title of Resource and Principal Investigator

Resource:
Laser Source of Intense Tunable Near-Ultraviolet Radiation

Principal Investigator:
John Calkins, Ph.D.
Room C-15
Department of Radiation Medicine
University of Kentucky Medical Center
800 Rose Street
Lexington, Kentucky 40536
(606) 233-5547

Services Available

Instruments: The principal instrument at this facility is the phase Model 1400 tunable dye laser. Accessory equipment is available to generate high-intensity UV continuously variable in wavelengths from 250-350 nm using frequency doubling. The primary beam 500-700 nm can also be used. Various items for calibration and monitoring of output power are included as well as general laboratory equipment for microassays, i.e., balances, incubators, etc., needed for action spectra studies using a variety of microorganisms.

- *Special Features:* The principal feature of this facility is the availability of the high-power density output (up to 500 w/m^2) throughout the operating range with very narrow bandwidth (less than 0.5 nm) and continuously tunable through the indicated range.
- *Service Personnel:* There are at present no full-time service personnel. However, the principal investigator and a staff of four part-time assistants are available to assist with new projects.
- *User Laboratory Space:* To be arranged depending on the needs of the proposed project.
- *User Contact Person:* John Calkins, Ph.D., (606) 233-5547

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Biological action spectra throughout the wavelength range of 250-350 nm. Useful for eukaryotic microorganisms and genetic endpoints as well as for other types of microorganisms.
- *Current Research:* Action spectra of prokaryotic microorganisms, genetic endpoints, and photosensitized reactions in both genetic toxicology and vascular microsurgery.
- *New Concept/Capability:* The principal use of this facility will be to compile UV-blue action spectra, where the need has long been recognized but the technology has been lacking. New concepts regarding photosensitized medical uses are under development.

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Title of Resource and Principal Investigator

Resource:
Extended Range Mass Spectrometric Biomedical Technology Resource

Principal Investigator:
Frank H. Field, Ph.D.
The Rockefeller University
1230 York Avenue
New York, New York 10021
(212) 570-8848

Services Available

- *Instruments:* High- and low-resolution MS: SRI Biospect gas chromatograph (GC)-chemical ionization quadrupole MS and VG Datasystems 2040 computer with capabilities for positive and negative chemical ionization and multiple ion monitoring; VG 70-250 GC/MS/Datasystem with capabilities for positive and negative electron ionization and chemical ionization and capillary GC; fission fragment (^{252}Cf) ionization MS; pulsed ion bombardment MS; chemical physics MS; Varian Aerograph Model 1400 Finnigan GC; Model 9600 GC; and Rainin HPLC.
- *Special Features:* The ^{252}Cf fission fragment mass spectrometer allows spectra to be obtained on compounds that are highly nonvolatile because of their size, polar nature, or ionic character. Examples are underivatized polypeptides, nucleotides, sulfonic acids, sulfonate salts, and similar compounds. The pulsed ion bombardment mass spectrometer provides very high sensitivity measurements on nonvolatile compounds.
- *Service Personnel:* Resource personnel perform analyses and assist in interpretation of results. Frequent users may be trained in instrument operation.
- *User Laboratory Space:* No user space provided.
- *User Contact Person:* Frank H. Field, Ph.D., (212) 570-8848.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This mass spectrometric resource can provide information about the identities and amounts of samples even when only very small amounts of material are available. Qualitative information can be provided on 10-100 ng or more of sample; quantitative information can be provided on 10-100 pg or more of sample. Samples can be pure compounds or complex mixtures. Using conventional mass spectrometric methods, materials with volatilities equal to or greater than those of tripeptides-pentapeptides can usually be handled. Using conventional mass spectrometric methods, materials with molecular weights below 800 can be handled routinely; those with molecular weights of 800-2,000 involve special handling and lower sensitivities. Ionization of materials is effected by electron ionization or chemical ionization, and with the latter, both positive and negative ions can be produced. The different kinds of ionization provide information about different aspects of the structure of a sample, and the type of ionization chosen depends on specific needs. Using the fission fragment mass spectrometer, involatile high molecular weight materials can be examined as mentioned above. Spectra on molecules with molecular weights in the range 5,000-10,000 daltons often can be obtained.
- *Current Research:* Development and utilization of fission fragment ionization mass spectrometry; development and utilization of fission fragment ionization methods for the detection and sequencing of peptides; development and utilization of pulsed ion bombardment mass spectrometry; development and utilization of thermospray ionization mass spectrometry; GC/MS analyses in the nanogram and subnanogram range; identification of unknown compounds; and quantitative GC/MS assays.
- *New Concept/Capability:* ^{252}Cf fission fragment spectrometry for nonvolatile samples. OH^- negative chemical ionization MS, pulsed ion bombardment mass spectrometry, and thermospray ionization mass spectrometry. These are methods for obtaining information about large, nonvolatile biological molecules.

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Biological Structure and Function: Mass Spectrometry

Title of Resource and Principal Investigator

Resource:
Resource for Biomedical Mass Spectrometry

Principal Investigator:
Dennis M. Bier, M.D.
Department of Medicine
Washington University
School of Medicine
660 South Euclid Avenue
St. Louis, Missouri 63110
(314) 362-6923

Services Available

• *Instruments:* Finnigan 3200 and 3300 quadrupole instruments with EI, CI, and FAB ionization (to mass 1,000) are available. Both packed and capillary column inlets are used as well as direct insertion inlets. An LKB-9000 low-resolution magnetic scanning GCMS also is available. Each GCMS has a dedicated data system. Both scanning and selected ion monitoring are available on each system. A nuclide 3-60 isotope ratio mass spectrometer automated and controlled by a DEC LSI 11/23 is also available. A VG ZAB SE with FAB ionization and collision cell for MS/MS analyses is expected to be operational in the fall of 1985.

The resource is nearing completion of the assembly and programming of a new DEC LSI 11/23-based mass spectrometry data system that operates under RSX-11M and utilizes readily available commercial peripherals. The system programs and construction plans will be available to qualified individuals.

• *Special Features:* Stable isotope enrichment measurement of molecules; addition of labeled compounds to physiological samples to serve as internal standards for high-precision, sensitive quantitation; stable isotopically labeled substrates as tracers for in vivo or in vitro metabolic and kinetic studies; methods for measuring stable isotope enrichment directly in metabolites from physiological samples via GCMS, and for measuring highly dilute tracer

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Research Emphasis or Application

Data Achievable with Resource's Methodologies: Quantitative analysis of biological compounds or drugs from physiological samples such as blood and tissue, using stable isotopically labeled internal standards and GCMS. Typical precisions are 1 percent on nanogram amounts; detection limits are usually at picogram amounts. Resource staff has extensive experience with extraction, derivatization, and measurement of a wide range of metabolites and drugs from a variety of physiological sample types. Measurement of stable isotope tracer enrichment for metabolic or kinetic studies in both precursor and product compounds. Stable isotopic enrichment can be measured to ± 0.1 atom percent using GCMS and ± 0.001 atom percent using isotope ratio mass spectrometry. The latter technique can measure ^{15}N, ^{13}C, ^{18}O for either N_2 or CO_2 gas samples obtained directly as expired air or converted via an automated process from ammonia or urea samples.

• *Current Research:* Stable isotope tracers to measure kinetic rates of glucose, amino acid, or fatty acid metabolism in humans; drug metabolism in newborns and children; diagnosis of patients with inborn errors of metabolism; FAB ionization analysis of substances to a molecular weight of 1,000 with emphasis on lipids.

• *New Concept/Capability:* Automated measurement of very high dilution ^{15}N, ^{13}C, or ^{18}O tracer enrichment via isotope ratio mass spectrometry in end products of metabolism or samples converted to CO_2 or ammonia. Measurement of high dilution ^{15}N or ^{13}C tracer enrichment directly in protein-derived amino acids and other metabolites via isotope-ratio-monitoring GCMS (under construction). Chiral analysis of carbohydrates by capillary GC and GCMS.

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Services Available

enrichment appearing in metabolic end products (CO_2, urea, or ammonia). Special emphasis is placed on the analysis of substrates in very small tissue samples and in the analysis of phosphorylated and other highly absorptive substrates.

- *Service Personnel:* Resource personnel maintain all instrumentation and assist and train routine users, who prepare their own samples and run their own analyses. Resource personnel are available to consult on methods development and data interpretation. Limited funds are available to defray travel and living expenses for visiting research scientists and trainees.
- *User Laboratory Space:* Wet-chemistry space available for external users to prepare samples prior to mass spectrometric measurement.
- *User Contact Persons:* Dennis M. Bier, M.D., (314) 362-6923; William R. Sherman, Ph.D., (314) 362-7015; and Dwight E. Matthews, Ph.D., (314) 362-7877.

Research Emphasis or Application

[1]Matthews, D. E., Ben-Galim, E., and Bier, D. M., *Analytical Chemistry* 51:80, 1979.
[2]Matthews, D. E., and Bier, D. M., Stable isotope methods for nutritional investigation. *Annual Review of Nutrition* 3:309, 1983.
[3]Leavitt, A. L., and Sherman W. R., Determination of inositol phosphates by gas chromatography. In *Methods in Enzymology* (Wood, W. A., ed.). Academic Press, 1982, pp. 9-18.
[4]Leavitt, A. L., and Sherman, W. R., Direct gas-chromatographic resolution of DL-myoinositol-1-phosphate and other sugar enantiomers as simple derivatives on a chiral capillary column. *Carbohydrate Research* 103:203-212, 1982.

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Biological Structure and Function: Mass Spectrometry

Title of Resource and Principal Investigator

Resource:
Biomedical, Bio-Organic, Mass Spectrometry Resource

Principal Investigator:
A. L. Burlingame, Ph.D.
Department of Pharmaceutical Chemistry
University of California
San Francisco, California 94143
(415) 666-5641
(Message) (415) 666-1225

Services Available

• *Instruments:* Kratos MS-25S double-focusing MS equipped for GC/MS operation with either glass capillary or packed columns; EI, and combined EI and CI ion sources with MID and direct-probe accessories and on-line to a Data General Nova II computer system. Kratos MS-50S double-focusing mass spectrometer with 23 kilogauss magnet, including EI, field desorption, xenon neutral (FAB), cesium ion liquid secondary ion mass spectrometry (SIMS) ion sources. MS-50S includes direct probe and fused silica capillary gas chromatographic inlet systems. The MS-50S is online to a real-time LOGOS-VAX 11/750 computer system. Waters and Altex high-pressure liquid chromatographs. WCOT glass capillary GC.

• *Special Features:* High-resolution glass capillary GC with both real-time resolution and high-resolution mass spectrometry; elemental composition chromatography (LOGOS-II HRMS software); exploratory field ionization sources, e.g., volcano; field-ionization kinetic (FIK) methods for studying unimolecular decomposition of metastable gaseous organic ions to picosecond time regions; and combined field desorption/electron impact (FD/EI) ion source for MS-50S with facility for collision-induced dissociation studies; positive and negative ion chemical ionization MS on MS-25S; positive and negative ion, cesium ion liquid-SIMS, on the MS-50S.

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Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* This resource provides analytical techniques for those investigators whose bioorganic and biomedical research requires identification of chemical structures, determination of individual components of mixtures, or qualitative molecular elemental and isotopic composition. Complete high-resolution mass spectra can be obtained using direct probe and GC inlets on sample sizes down to 10 ng. Quantitative analysis of biological substances, such as drugs and their metabolites, can be accomplished on sample sizes down to the order of 10^{-12} g. Also available are both field desorption and liquid-matrix secondary ion sputtering techniques that permit studies of new classes of compounds of biological and clinical interest. This methodology is well-suited to study nonvolatile chemically and thermally labile polar substances that may be isolated from biological media and purified by techniques such as high-pressure liquid chromatography. Currently, a molecular weight range up to 10,000 daltons is under active investigation. One class of structural problems amenable to field desorption and liquid-SIMS sputtering techniques is the binding of carcinogens and drugs to DNA and to the biopolymers. A second class is the post-translational modifications of proteins and peptides as well as tyrosine phosphate and sulfate and other modified proteins. The third class is higher molecular weight oligosaccharides, glycolipids, and glycoconjugates in general.

• *Current Research:* Urinary steroids; vitamin D metabolism; bile salt and phospho- and glycolipid metabolism; drug metabolites; high-resolution, field desorption (FD), liquid matrix-SIMS for determination of biomolecular structure, e.g., carcinogens and drugs covalently bound to cellular macromolecules, polar drug conjugates, etc; and structural studies of high-molecular weight peptides, oligosaccharides, and heterobiopolymers.

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Services Available

- *Service Personnel:* Collaborators and students are trained to run Kratos MS-25S with data processing system. Probe HRMS, HRGC/HRMS analyses, field desorption, and xenon neutral and cesium ion liquid-SIMS run by resource personnel. However, interactive selection, manipulation, and interpretation of high resolution, field desorption, and secondary ion mass spectral data are carried out by the visiting investigator using the LOGOS-VAX computer system. Training for collaborators and occasional short courses in MS in bioorganic and biomedical problems. Graduate level course in MS in life sciences in department of pharmaceutical chemistry (PC235).
- *User Laboratory Space:* Limited user laboratory space available when required.
- *User Contact Persons:* A. L. Burlingame, Ph.D.; Arnold M. Falick, Ph.D.; J. O. Whitney, Ph.D.; M. F. Schwartz, (415) 666-2582.

Research Emphasis or Application

- *New Concept/Capability:* Recent development of computerized FDMS and cesium ion liquid matrix-SIMS permits the analysis of free highly polar compounds and salts of biological origin, which have not been amenable to investigation by conventional MS techniques requiring derivatization to effect volatility. Furthermore, the collision-induced dissociation (CID) techniques may be employed in conjunction with FDMS and SIMS to generate structurally informative fragmentation patterns for use in structural elucidation of polar and/or thermally labile compounds up to 3,000 daltons. In addition, vigorous development is under way to deal with biological substances up to 10,000 daltons.

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Title of Resource and Principal Investigator

Resource:
Clinical Mass Spectrometry Resource

Principal Investigator:
Paul V. Fennessey, Ph.D.
University of Colorado Health Sciences Center
School of Medicine
Departments of Pediatrics and Pharmacology
4200 East 9th Avenue
Mail Stop #C232
Denver, Colorado 80262
(303) 394-8196

Coinvestigator:
Robert C. Murphy, Ph.D.
(303) 394-8962

Services Available

- *Instruments:* A VG Micromass MM-16 with electron impact and chemical ionization source, capillary and packed gas chromatographic inlets, and a foreground/background data system. A Finnigan 3200 with electron impact source, gas chromatograph, and data system. A VG Micromass 7070E-HF high-resolution mass spectrometer fitted for EI/CI and FAB ionization. Medium mass capability m/7 2600 at full acceleration potential of 6 kV.
- *Special Features:* Stable isotope analysis with emphasis on clinical studies. These include the quantitative analysis of ^{2}D, ^{13}C, ^{15}N, and the metals zinc, copper, and iron. Central data processing unit for special (i.e., isotope cluster) and routine searchers. Summer course to be offered for interested investigators. Fast atom bombardment (FAB) mass spectrometry techniques available for complex, polar molecules.
- *Service Personnel:* Instruments and data system operation carried out by facility personnel. Users are encouraged to participate in the analysis and interpretation of data.
- *User Laboratory Space:* No user space is provided.
- *User Contact Person:* Paul V. Fennessey, Ph.D., (303) 394-8196.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Quantitative analysis of natural metabolites found in physiological fluids. Separation, identification, and quantitation of chemically different classes of compounds (e.g., prostaglandins, organic acids, steroids, CNS compounds, etc.).
- *Current Research:* New methods for the synthesis of internal standards for the quantitative analysis of biologically important compounds. Identification of steroid profiles and steroid biotransformations in normal individuals and in patients with biochemical (inborn errors) and pathological irregularities. Measurement of the dynamics of the metabolism of important biochemicals such as sugars, steroids, etc. Study of leukotriene biosynthesis and regulation leading to the synthesis of slow reacting substance of anaphylaxis. Effect of ethanol and neuroleptics on biogenic amine synthesis in the central nervous system.
- *New Concept/Capability:* Isolation and quantitative analysis of trace compounds of clinical interest. FAB of complex biomolecular compounds associated with clinical medicine.

[1]Pike, A. W., Moynihan, C. M., Kibler, S., Marsh, P. G., and Fennessey, P. V., Semi-micro quantitative analysis of complex urinary steroid mixtures in healthy and diseased states. *Journal of Chromatography* 306:39-50, 1984.
[2]Christie, D. B., Hall, M., Moynihan, C. M., Hambridge, K. M., and Fennessey, P. V., Quantitative analysis of bioactive trace metals and trace metal stable isotopes using mass spectrometric techniques. P. G. Johnson and J. R. Turnland (eds.). In *ACS Symposium Series: Stable Isotopes in Nutrition*, 1984.
[3]Clay, K. L., Stene, D. O., and Murphy, R. C., Analysis of AGEPC by fast atom bombardment mass spectrometry. *Biomedical Mass Spectrometry* 11:47-49, 1984.
[4]Horowitz, K. B., Mockus, M. B., Pike, A. W., Fennessey, P. V., and Sheridan, R. L., Progesterone receptor replenishment in T47D human breast cancer cells: Roles of protein synthesis and hormone metabolism. *Journal of Biological Chemistry* 258:7603-7610, 1983.

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Research Emphasis or Application

[5]Hunter, S. W., Murphy, R. C., Clay, K., Goren, M. B., and Brennan, P. J., Trehalose-containing lipooligosaccharides: A new class of species-specific antigens from mycobacteria. *Journal of Biological Chemistry* 258:10481-10487, 1983.
[6]Matz, S. A., Murphy, R. C., and Fujimoto, W. Y., Effect of glucose-induced release of lipoxygenase-mediated metabolites of arachidonic acid. *Diabetes* 33:119-124, 1984.
[7]Clay, K. L., Murphy, R. C., Andres, J. L., Lynch, J., and Henson, P. M., Structure elucidation of platelet activating factor derived from human neutrophils. *Biochemical and Biophysical Research Communications* 121:815-825, 1984.

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Biological Structure and Function: Mass Spectrometry

Title of Resource and Principal Investigator

Resource:
Mass Spectrometry Facility for Biomedical Research

Principal Investigator:
Klaus Biemann, Ph.D.
Massachusetts Institute of Technology
Department of Chemistry
Cambridge, Massachusetts 02139
(617) 253-7221

Services Available

- *Instruments:* High-resolution mass spectrometers CEC 21-110B and Varian MAT 731, both with photoplate capability; photoplate reader coupled to computer. VAX-11/750-based computer system linked to satellite PDP-11 microprocessor-based data systems on mass spectrometers. Complete array of mass spectrometry software including comparison against reference standards. Expected June 1985: JEOL HX-110/HX-110 four-sector extended mass-range, high-resolution mass spectrometer with PDP 11/73-based DA-5000 data system.
- *Special Features:* Fast atom bombardment and field desorption measurements; determinations of heavy isotope incorporation and other specialized MS applications; new hardware and software development. Also offer microfilmed data and reconstructions and measurement of photoplates.
- *Service Personnel:* Instrument and computer operation are performed by facility personnel. Users are encouraged to be present for special sample manipulation or for observation.
- *User Laboratory Space:* No user space provided (exceptions can be arranged).
- *User Contact Persons:* Catherine E. Costello, Ph.D., (617) 253-7224; W. Rodney Mathews, Ph.D., (617) 253-7229.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Identification or structure determination of complex biological, organic, and organometallic molecules with molecular weights up to 2,000 by electron ionization, high-resolution mass spectrometry for compounds that are sufficiently volatile (vapor pressure, 10^{-6} torr at 250°C) and thermally stable, and/or fast atom bombardment and field desorption mass spectrometry for less volatile or thermally unstable substances. Extended mass range also available. Identification of the components of mixtures (if amenable to gas chromatography) by GC/MS. Special computer software is available to investigate very complex mixtures.
- *Current Research:* Molecular structure analysis (marine natural products, enzymes, inorganic complexes, peptides); protein sequencing and techniques and applications of fast atom bombardment and field desorption mass spectrometry.
- *New Concept/Capability:* Fast atom bombardment is now available to supplement field desorption as an ionization method for nonvolatile and thermally unstable samples. Useful in both positive and negative ionization modes; capable of providing exact mass measurements for determination of elemental composition of ions. Choice of methods varies with sample. Expected June 1985: Installation of new instrument (JEOL HX-110/HX-110) with mass range over 12,000 daltons (and beyond, at reduced sensitivity). Four-sector geometry will provide high-resolution MS/MS capability and decompositions activated by collisions. Electron ionization, chemical and direct chemical ionization, fast atom bombardment, and field desorption will be available, each in both positive and negative ionization modes.

[1]Gibson, B. W., and Biemann, K., Strategy for the mass spectrometric verification and correction of the primary structures of proteins deduced from their DNA sequences. *Proceedings of the National Academy of Sciences* 81:1956-1960, 1984.

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Research Emphasis or Application

[2]Martin, S. A., Karnovsky, M. L., Krueger, J. M., Pappenheimer, J. R., and Biemann, K. Peptidoglycans as promoters of slow-wave sleep: I., Structure of the sleep-promoting factor isolated from human urine. *Journal of Biological Chemistry*, In press.

[3]Waslyk, J. M., Biskupiak, J. E., Costello, C. E., and Ireland, C. M., Cyclic peptide structure from the tunicate *Lissoclonum patella* by FAB mass spectrometry. *Journal of Organic Chemistry* 45:4445-4449, 1983.

[4]Abrams, M. J., Davison, A., Jones, A. G., Costello, C. E., and Pang, H., Synthesis and characterization of hexakis alkyl and isocyanate complexes of technetium(I). *Inorganic Chemistry* 22:2798-2800, 1983.

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Biological Structure and Function: Mass Spectrometry

Title of Resource and Principal Investigator

Resource:
Michigan State University Mass Spectrometry Facility

Principal Investigator:
J. Throck Watson, Ph.D.
Department of Biochemistry
Michigan State University
East Lansing, Michigan 48824
(517) 353-0855

Coinvestigators:
John F. Holland, Ph.D.
(517) 353-6743
Christie G. Enke, Ph.D.
(517) 353-4511
John Allison
(517) 353-4511
Charles C. Sweeley
(517) 353-3257

Services Available

- *Instruments:* High-field, high-mass, double-focusing MS with EI/FAB combination ion source and high-resolution capability in peak matching mode; LKB 9000 single-focusing magnetic sector MS; two Hewlett-Packard 5985 quadrupole GC/MS computer systems with combination EI and CI sources capable of packed and capillary column GC; LKB 2091 GC/MS with EI source and capillary column inlet; Extranuclear Labs triple quadrupole mass spectrometer; and CVC time-of-flight mass spectrometer. Distributed computing network with dedicated front-end systems consisting of DEC PDP 8/e and H/P 21MX disk-based computer systems and a DEC PDP 11/44 host system with the resident executive program of RSX-11M. Real-time, interactive, and offline remote access MS data processing.
- *Special Features:* Fast atom bombardment MS; quantitative metabolic profiling; computer-controlled selected ion monitoring for micro-analysis of multicomponent samples; and production of mass chromatograms from arrays of mass spectra.
- *Service Personnel:* Facility personnel perform all system operations, and training is available for investigators.
- *User Laboratory Space:* No user space provided.
- *User Contact Person:* Brian D. Musselman, facility manager, (517) 353-0612.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The components of physiological fluid mixtures such as acidic metabolites, steroids, and other molecules of biological importance can be identified and quantitated automatically by a reverse library search routine applied to GC/MS data. To assist the clinician, the data are presented in terms of standard deviations from established norms, in formats amenable to rapid visual examination. Profiles can be used to follow trends of individual metabolic states in relation to time-dependent parameters or to examine differences in subject groups under similar testing circumstances. Fragmentation pathways can be investigated with a triple quadrupole mass spectrometer (TQMS); in turn, characteristic fragmentation pathways stimulated by collisionally activated decomposition (CAD) and measured by TQMS can be used to detect specific compounds in complex mixtures. Polar nonvolatile compounds can be analyzed by fast atom bombardment (FAB).
- *Current Research:* Development of new instrumental approach to MS/MS by combining time-of-flight and momentum analysis of mass; development of a time-array detector for a time-of-flight mass spectrometer; refinement of distributed computing and techniques for data processing of mass spectra; study of fragmentation mechanisms by MS/MS; and development of quantitative methodology based on MS/MS and GC-MS/MS.
- *New Concept/Capability:* Modification of a conventional magnetic mass spectrometer with a pulsed source and time-of-flight detector provides an inexpensive unit-resolution approach to MS/MS methodology. Time-array detection in time-of-flight mass spectrometry will permit all ions from each pulse of the ion source to be detected, thereby improving ion statistics, particularly for chromatographic conditions that place increasing demands on scan speed and spectrum generation frequency. Sophisticated reverse library searching computer programs have been written that enable the automatic identification and quantitation of components present in complex biological mixtures. A reference library consisting of qualitative and quantitative mass spectral data for more than 400 urinary acid and steroid metabolites has been compiled for routine use. Different concepts of statistical analysis are being employed to maximize the intelligence gained from the automated analysis of the GC-MS data files.

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

Resource:
National NMR Facility for Biomolecular Research

Principal Investigator:
Leo J. Neuringer, Ph.D.
Molecular Biophysics Group
Francis Bitter National Magnet Laboratory
Massachusetts Institute of Technology
170 Albany Street
Cambridge, Massachusetts 02139
(617) 253-5592

Services Available

• *Instruments: —Spectrometer A:* Wide-bore (8.9 cm) high-resolution (360 MHz), FT spectrometer to observe ^{1}H (10 mm), ^{2}H (20 mm), ^{13}C (20 mm), ^{15}N (20 mm), ^{19}F (10 mm), and ^{31}P (20 mm) in liquid samples.

—Spectrometer B: Wide-bore (8.9 cm) high-frequency (400 MHz), FT research spectrometer for high-resolution studies of solids and membranes, using double resonance, cross polarization, and magic-angle sample spinning techniques, multinuclear (^{1}H, ^{2}H, ^{13}C, ^{14}N, ^{15}N, ^{19}F, ^{31}P).

—Spectrometer C: High-resolution (500 MHz) FT apparatus with versatile pulse programmer, observe capability for ^{1}H and ^{2}H.

—Spectrometer D: High-frequency (320 MHz) FT spectrometer utilizing a wide-bore (10 cm) magnet system for solid-state studies. Any type of double-resonance, multiple-pulse, and magic-angle sample spinning experiment can be performed. Nuclei available: ^{1}H, ^{2}H, ^{13}C, ^{14}N, ^{15}N, ^{17}O, ^{19}F, and ^{31}P.

—Spectrometer E: Under construction is a high-resolution 600 MHz multinuclear FT spectrometer with a persistent mode 14.1 tesla magnet system (5.1-cm bore). Capability of performing any NMR experiment in liquid- or solid-state spectroscopy.

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Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* The resource makes available high-field NMR spectrometers to biomedical investigators who need to study biological molecules (liquid- or solid-state), viral particles, cells, or intact tissues, and small animals under conditions of maximal spectral dispersion (resolution) with enhanced sensitivity. It is possible to use the spectrometers as analytic instruments to identify the type of compound present (e.g., mineral phases of bone) and to determine the type of metabolites and their state (labile or immobile) within. Information concerning the structure of a given compound in the solid state or its conformation in the liquid state can be obtained. The site of interaction of two molecules can be characterized; for example, drug-DNA binding. A complete repertoire of pulse sequences also makes it feasible to obtain motional information about biomolecules.

• *Current Research:* Natural and model membrane structure, dynamics, function, phase diagrams, phase transitions, and solid-state studies of amino acids, peptides, and bone; protein conformation and interactions, e.g., insulin, lac repressor, lambda repressor; dynamics of energy metabolism in skeletal muscle, cells, animal brain, human eye lens; repressor-DNA operator structure and dynamics; DNA structure and dynamics.

• *New Concept/Capability:* The major new capability will be the observation of protons at 600 MHz under conditions of high resolution, using Fourier transform techniques. This advanced spectrometer was designed, constructed, and tested at this laboratory and is nearing operational status. This instrument will enable the study and characterization of biological molecules using NMR spectroscopy with maximal spectral resolution and enhanced sensitivity. The versatile pulse programmer will permit performance of all types of double-resonance and relaxation time studies important to investigations of biological materials. A new multinuclear FT spectrometer console replaced the Bruker unit on Spectrometer A in 1984. A new 8.4T/8.9 cm magnet system is available for studies of metabolism in living systems. It is planned to create a photo-CIDNP capability for observation of proton resources at 360 and 500 MHz.

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Biological Structure and Function: Nuclear Magnetic Resonance

Services Available

- *Special Features:* 500 MHz spectra, solid-state spectra, saturation and inversion transfer techniques for ^{31}P; variable temperature; stations for off-line plotting and data processing; two-dimensional FT NMR; techniques for detection of ^{15}N nuclei with enhanced sensitivity.
- *Service Personnel:* Users are trained to run their own spectra. Consultation is available and collaborative research with staff is possible.
- *User Laboratory Space:* User space available for limited onsite sample manipulations; pH meter, microbalance, centrifuge, magnetic stirrers, some glassware. Users provide their own NMR tubes, solvents, and chemicals.
- *User Contact Person:* David J. Ruben, Ph.D., (617) 253-5598.

Research Emphasis or Application

[1]Aue, W. P., Ruben, D. J., Griffin, R. G., *Journal of Chemical Physics* 80:1729, 1984.
[2]Cheshnovsky, D., Neuringer, L. J., Williamson, K. L., *Journal of Protein Chemistry* 1:335, 1983.
[3]Patel, D. J., Ikuta, S., Kozlowski, S. A., Itakura, K., *Proceedings of the National Academy of Sciences* 80:2134, 1983.
[4]Weiss, M. A., Patel, D. J., Sauer, R. T., Karplus, M., *Proceedings of the National Academy of Sciences* 81:130, 1984.
[5]Lu, P., Cheung, S., Arndt, K., *Journal of Biomolecular Stereodynamics* 1: 509, 1983.

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

Resource:
NMR Facility for Biomedical Studies

Principal Investigator:
Aksel A. Bothner-By, Ph.D.
MPC Corporation
4400 Fifth Avenue
Pittsburgh, Pennsylvania 15213
(412) 578-3149

Services Available

- *Instruments:* Two high-field multinuclear spectrometers interfaced with a 140.9 kG magnet, allowing observation of protons at 600 MHz, phosphorus at 243 MHz, deuterium at 92 MHz, ^{27}Al at 156 MHz, ^{11}B at 192 MHz, and other nuclei at corresponding frequencies. One spectrometer is interfaced with an XDS Sigma 5 computer for accumulation and digital processing of spectral data in the correlation mode. The second is a modified Bruker console for observation in the FT mode.
- *Special Features:* Correlation spectroscopy and Fourier transform spectroscopy, including many varieties of 2-DFT, are available. Capabilities for homo- or heteronuclear double irradiation and the precise observation of longitudinal or transverse nuclear Overhauser effects are installed. Spectra may be accumulated under automatic control. Variable temperature proton spectra. MAS solids studies of ^{27}Al and ^{11}B. Pulsed gradient diffusion studies.
- *Service Personnel:* Facility personnel operate instruments for new users; training provided in operation and interpretation of results for long-term users.
- *User Laboratory Space:* Space and facilities available for sample preparation, determination of pH, UV-visible spectroscopy, etc.
- *Service Fees:* Basic fee $39/hour on working days and $19.50/hour on nights and holidays. Waiver of fees for exploratory studies and collaborative studies.
- *User Contact Person:* P. K. Mishra, (412) 578-3161.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource provides NMR spectra of samples in solution at the highest field available. The spectra are useful in determining chemical structure and conformation of complex molecules and provide information on dynamic processes as well as static structure. Correlation spectroscopy is especially useful in the observation of weak solute peaks in the presence of strong solvent peaks, such as those arising from water or methanol. Fourier transform spectroscopy is useful for the study of relaxation processes, dynamic nuclear Overhauser effects, transverse Overhauser effects, and 2-D NMR spectroscopy.
- *Current Research:* Transverse Overhauser effects;[1] magnetic field orientation effects in the spectra of small molecules;[2] conformation studies of ion-binding cyclic peptides; interaction between DNA and model proteins; mechanism of oxygenation of hemoglobin and mutant hemoglobins; dynamics of phospholipids in cell membranes, conformations of peptides (bradykinin, gramicidin S, etc.)
- *New Concept/Capability:* The 140.9 kG magnet provides the greatest spectral dispersion of any instrument now available and is useful in dissecting and assigning spectral regions that display severe overlap of signals at lower fields. In addition, the strength of the field is sufficient to partially orient small molecules and produce observable effects in the NMR spectrum. These are useful in determining geometric structure and electronic properties.

[1]Bothner-By, A. A., Stephens, R. L., Lee, J. M., Warren, C. D., and Jeanloz, R. W., Structure determination of a tetrasaccharide: Transient nuclear Overhauser effects in the rotating frame. *Journal of the American Chemical Society* 106:811, 1984.
[2]Bothner-By, A. A., Gayathri, C., Van Zyl, P. C. M., and MacLean, C., Dipolar magnetic field effects in NMR spectra of liquids. *Chemical Physics Letters* 87:192, 1982.

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Title of Resource and Principal Investigator

Resource:
NMR Imaging and Spectroscopy in Vivo Resource

Principal Investigator:
Katherine N. Scott, Ph.D.
Associate Professor
Department of Radiology
University of Florida
Box J 374
J. Hillis Miller Health Center
Gainesville, Florida 32610
(904) 392-3010

Coinvestigators:
E. Raymond Andrew, Ph.D., FRS
(904) 392-8006
Jeffrey R. Fitzsimmons, Ph.D.
(904) 392-8002
Thomas H. Mareci, Ph.D.
(904) 392-8005

Services Available

• *Instruments:*
—*Spectrometer/imager:* 2 T, 23.5-cm clear-bore multinuclear system by General Electric Company NMR Instruments with Oxford Instruments Model 80/310 HR high-field homogeneity superconducting magnet. The console is a Nicolet NT-80 and the image display controller is a Cromemco Z-2D. The system is a versatile multinuclear (^{1}H, ^{31}P etc.) spectrometer with full imaging and spectral localization capability.

—*Whole body imager:* 0.15 T Technicare Teslacon ^{1}H imaging system with versatile technique selection, multislice, multiecho capability. Cardiac gating, bandwidth reduction, conventional and out-of-phase imaging with a variety of coil designs are available.

—*Small-scale spectrometer/imager:* Nicolet model NB-300, 7.1 T, 2.5-cm bore multinuclear FT spectrometer modified to provide limited imaging and spectral localization capability (^{1}H, ^{31}P, ^{13}C). The spectrometer has retained full multinuclear, double-resonance, 2-D FT capability.

—*Small-scale spectrometer/imager:* Bruker model HX-90, 2.1 T, 2.5-cm magnet gap, multinuclear FT spectrometer modified to provide imaging and spectral localization capability (^{1}H and ^{31}P).

• *Special Features:* The whole-body imager has several specialized receiver coils that were developed in-house for imaging the neck, knee, back, abdomen, eye, face, etc. All the spectrometer/imagers have multinuclear, double-resonance, FT spectroscopy capability. The

Continued on next page

Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* ^{1}H whole body imaging at 0.15 T with multislice, multiecho, cardiac gating, bandwidth reduction, conventional vs. out-of-phase imaging. 2 T NMR of small animals and of human arms/legs: ^{1}H imaging and chemical shift imaging, localized spectroscopy (^{1}H, ^{31}P, ^{13}C) T_1 and T_2 measurements. 2.1 T and 7.1 T: ^{1}H and ^{31}P NMR of perfused organs from small animals, tissue samples, tumors on rat/mouse tails. On all systems: comparison of imaging, spectral localization, and spectroscopy techniques.

• *Current Research:* This resource specializes in developing techniques and instrumentation for imaging and in vivo spectroscopy. Current research interests are better localization techniques for spectroscopy,[1,2,3] image signal-to-noise enhancement,[4] tissue contrast enhancement,[5] relaxation measurements in vivo,[6] and rf coil development.[7] Biological applications are concerned with clinical ^{1}H imaging and with ^{1}H and ^{31}P in vivo spectroscopy in tumor detection and tumor response to therapy.

• *New Concept/Capability:* In vivo NMR spectroscopy can noninvasively monitor biochemical processes. Our research over the past few years has led to better localization techniques by the sensitive point method and by pulsed field gradients with selective Fourier transformation.[2,3] Our rf coil design,[7] image signal-to-noise,[4] and contrast[5] enhancement techniques yield some of the best images from a 0.15 T resistive system.

[1]Scott, K. N., Brooker, H. R., Fitzsimmons, J. R., Bennett, H. F., and Mick, R. C. Spatial localization of ^{31}P nuclear magnetic resonance by the sensitive point method. *Journal of Magnetic Resonance* 50:339, 1982.

[2]Mareci, T. H., and Brooker, H. R., High-resolution magnetic resonance spectra from a sensitive region defined with pulsed field gradients. *Journal of Magnetic Resonance* 57:157, 1984; Brooker, H. R. and Mareci, T. H., Selective Fourier transformation localization of high-resolution magnetic resonance spectra. *Magnetic Resonance in Medicine.* In press.

[3]Mareci, T. H., Thomas, R. G., Scott, K. N., and Brooker, H. R., Combination pulse gradient and surface coil localization of chemical shift resolved spectra. Abstracts, *Third Annual Meeting of the Society of Magnetic Resonance in Medicine* 494, 1984.

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Services Available

Bruker HX-90 has high spatial resolution (0.1 mm) ^{1}H imaging capability. The 2 T spectrometer/imager has imaging, chemical shift imaging, and state-of-the-art spectral localization capability. Workshops on imaging and in vivo spectroscopy are offered three times per year.

- *Service Personnel:* Resource staff includes NMR physicists, chemists, biochemists, spectroscopists, and instrument designers. The particular expertise of the staff is in pulse and imaging techniques, rf coils, and instrument design. Collaboration with staff members is encouraged. All long-term users must provide their own operators who require minimal assistance. Training and consultation are provided.
- *User Laboratory Space:* The Health Center has complete animal care, preparation, and boarding facilities. Laboratory space and equipment for routine sample preparation are available. The resource supplies all materials related to the instrument; the user supplies all animals and materials related to sample preparation. Office space is provided for extended visits.
- *User Contact Person:* Katherine N. Scott, Ph.D., (904) 392-8010.

Research Emphasis or Application

[4]Fitzsimmons, J. R., Mick, R. C., and Mareci, T. H., Image enhancement on a 0.15 T resistive system. Abstracts, *Third Annual Meeting of the Society of Magnetic Resonance in Medicine,* 1984.

[5]Scott, K. N., and Mareci, T. H., Enhanced fat-water contrast imaging at 0.15 T. Abstracts, *Third Annual Meeting of the Society of Magnetic Resonance in Medicine* 688, 1984; *Magnetic Resonance in Medicine,* submitted.

[6]Sattin, W., Fitzgerald, L. T., Mareci, T. H., and Scott, K. N., Determination of T_1 by NMR imaging in an inhomogenous rf field. Abstracts, *Third Annual Meeting of the Society of Magnetic Resonance in Medicine* 654, 1984.

[7]Fitzsimmons, J. R., Thomas, R. G., and Mancuso, A., Proton imaging with surface coils on a 0.15 T resistive system. *Magnetic Resonance in Medicine,* submitted.

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

Resource:
Purdue University Biochemical Magnetic Resonance Laboratory

Investigators:
Robert L. Van Etten, Ph.D.
(Biochemical Applications)
John L. Markley, Ph.D.
(Biochemical Applications)
Robert E. Santini, Ph.D.
(Instrumental Developments)
William M. Westler, Ph.D.
(Operations)
Department of Chemistry
Purdue University
West Lafayette, Indiana 47907
(317) 494-5287

Services Available

• *Instruments:*
—*Narrow-Bore Spectrometer:* Nicolet NT-470 high-resolution spectrometer system with Oxford Instruments 11.1 T superconducting solenoid (470 MHz ^{1}H NMR). *Probes:* ^{1}H (5 mm), ^{2}H (8 mm), ^{13}C (8 mm), ^{31}P (8 mm), and ^{15}N (8 mm). *Modes of operation:* pulse Fourier transform; high-power or low-power (Gueron or Redfield "2,1,4"); rapid scan correlation (Dadok and Sprecher); quadrature detection; pulsed field gradient. *Decoupling:* single- or multiple-frequency ^{1}H and broadband (WALTZ) ^{1}H; computer control of pulse level (bilevel decoupling), phase, frequency, and timing. *Probe temperature:* variable temperature (−80°C to +150°C) under computer control for all probes. *Computer system:* Nicolet 1180 computer, 32 K data memory, 293B pulse programmer, CDC dual, high-density disk, high-speed paper punch, digital plotter, and NTCFTB software. *Photoprobe and laser:* ^{1}H (5 mm) photoprobe; 8-watt argon ion laser (Coherent Innova); shutter under computer control.
—*Wide-Bore NMR Spectrometer:* Nicolet NT-200 high-resolution spectrometer system with Oxford Instruments 4.7 T superconducting solenoid (200 MHz ^{1}H NMR). *Probes:* ^{1}H (5 mm, 12 mm), ^{13}C (12 mm, 20 mm), and ^{31}P (20 mm). *Modes of operation:* pulse Fourier transform, low-power ^{1}H and ^{31}P rapid scan correlation; quadrature detection. *Decoupling:* single frequency ^{1}H and broadband (WALTZ) ^{1}H; computer control of pulse level (bilevel decoupling), phase, frequency, and spacing. *Probe temperatures:* variable temperature (−80°C to +150°C) under computer control for

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Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* This resource provides instrumentation, instruction, advice, and collaboration opportunities to biomedical investigators whose research requires high-field or wide-bore multinuclear NMR spectroscopy. Some present users are investigating structure-function relationships in peptides, proteins, nucleic acids, carbohydrates, lipids, and other complex biomolecules.[1] Others use NMR to deduce the structures of natural products, to monitor the products of chemical syntheses, or to determine concentrations of metabolites in living cells, tissues, organs, or intact organisms under varying physiological conditions.[2,3]

• *Current Research:* New methods and applications of two-dimensional Fourier transform NMR spectroscopy and effective processing, storage, and presentation of spectra. Areas of investigation include: active sites of enzymes, mechanisms of interactions between enzymes and inhibitors, protein-nucleic acid interactions, mechanisms of electron transport in photosynthesis, and dynamics of virus particles.[4,5] Techniques in use include: isotopic labeling of biomolecules, analysis of pH titrations by NMR, chemical modification of proteins, and low-temperature spectroscopy.

• *New Concept/Capability:* Heteronuclear (^{13}C, ^{1}H) two-dimensional Fourier transform NMR spectroscopy of proteins leads to a useful simplification of complex spectral regions and direct cross-assignment of ^{13}C and ^{1}H resonances.[6] The ^{18}O isotope shift of ^{13}C NMR peaks can be used to obtain information about the reactions involving carbon-oxygen bond-breaking and/or bond-forming processes, as has been demonstrated in the acid and enzymatic hydrolysis of oxiranes.[7]

[1]Gorenstein, D. G., ^{31}P NMR of transfer ribonucleic acids. In *P-31 NMR: Principles and Applications*, D. G. Gorenstein (ed.), pp. 265-298, 1984.
[2]Richard, J. P., and Frey, P. A., Stereochemical course of phosphoanhydride synthesis. *Journal of the American Chemical Society* 105:6605-6609, 1983.
[3]Barany, M., Doyle, D. D., Graff, G., Westler, W. M., and Markley, J. L., Natural abundance ^{13}C NMR spectra of human muscle, normal and diseased. *Journal of Nuclear Magnetic Resonance in Medicine* 1:30-43, 1984.

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Biological Structure and Function: Nuclear Magnetic Resonance

Services Available

all probes. *Computer system:* Nicolet 1180 computer, 239A' pulse programmer, 32 K data memory; CDC dual, high-density disk; digital plotter; and NTCFT software.

—*Laboratory Equipment:* Desk centrifuge (Brinkman 3200), pH meter (Corning 112), balance (Mettler H6T), refrigerator/freezer; variable-temperature bath.

—*Special Features:* Staff teaches new operators during their initial visits; biennial workshop on biochemical NMR; an off-line computer system that duplicates all functions of the data system on each instrument is available for data reduction, manipulation, training, and software development. Staff is experienced in homonuclear and heteronuclear two-dimensional NMR methods and laser photo CIDNP experiments.

- *Service Personnel:* Qualified investigators are encouraged to use the spectrometer independently; use with the assistance of one staff member is available at particular times; service spectroscopy is provided for limited numbers of routine samples.
- *User Laboratory Space:* Chemical bench and desk space are provided for outside users during their stay in the laboratory. Users provide their own NMR tubes, solvents, and chemicals.
- *Scheduling:* Requests for short-term use are reviewed monthly. Long-term users require approval of the advisory committee. The schedule is made up on the 20th of the month for the following month.
- *User Contact Person:* Jeanne M. Winstead, operations assistant (317) 494-5283; NMR Laboratory, (317) 494-5287; Instrumentation Laboratory, (317) 494-5230.

Research Emphasis or Application

[4]Chan, T.-M., Nuclear magnetic resonance studies of two-iron-two sulfur ferredoxins. 3. Heteronuclear (^{13}C, ^{1}H) two-dimensional NMR spectra, ^{13}C peak assignments, and ^{13}C relaxation measurements. *Biochemistry* 22:5996-6002, 1983.

[5]Ulrich, E. L., John, E. M.-M., Gough, G. R., Brunden, M. J., Gilham, P. T., Westler, W. M., and Markley, J. L., Imino proton assignments in the ^{1}H NMR spectrum of the lambda phage O_R3 DNA fragment. *Biochemistry* 22:4362-4365, 1983.

[6]Westler, W. M., Ortiz-Polo, G., and Markley, J. L., Two-dimensional ^{1}H-^{13}C chemical shift correlated spectroscopy of a protein at natural abundance. *Journal of Magnetic Resonance* 58:354-357, 1984.

[7]Risley, J. M., Kuo, F., and Van Etten, R. L., ^{18}O isotope effect in ^{13}C nuclear magnetic resonance spectroscopy. 7. Hydrolysis of 2,2-dimethyloxirane in dilute acid and by microsomal epoxide hydratase. *Journal of the American Chemical Society* 105:1647-1652, 1983.

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

Resource:
Regional Resource for NMR in Vivo Spectroscopy

Principal Investigator:
John S. Leigh, Jr., Ph.D.
Biochemistry/Biophysics
505 Goddard Laboratories/G4
University of Pennsylvania
Philadelphia, Pennsylvania 19104
(215) 898-8033

Coinvestigators:
Britton Chance, Ph.D.
(Coprincipal Investigator)
(215) 898-4342
Alan McLaughlin, Ph.D.
(Research Director)
(215) 898-8033
Donald Younkin, M.D.
(Clinical Director)
(215) 596-9200

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Services Available

- *Instruments:* Available by January 1985: a 1-meter bore 1.5 Tesla NMR magnet giving ^{1}H at 64 MHz; ^{31}P at 26 MHz with versatile spectrometer for multinuclear spectroscopy and imaging; specialized probes for various nuclei. Available by January 1986: Separate data analysis station with 1 Mbyte memory expansion and array processor for 2-D imaging. Electronic equipment: RF bridge, sweep generator, and monitoring scope for probe tuning; Physiological monitoring equipment: transcutaneous tissue oxygen electrode, tissue potassium electrode, tissue O_2 and CO_2 electrodes, amplifier, electrometer, laser Doppler blood flow meter, hemoglobinometer.
- *Special Features:* By June 1985, the system will be capable of metabolic spectroscopy including (but not limited to) ^{1}H, ^{13}C, ^{15}N, ^{23}Na, and ^{31}P; it will be equipped for physiological synchronization (gating) and decoupling, chemical shift resolved metabolic imaging, magnetization transfer experiments, high-resolution proton imaging/T_1T_2 flow, 2-D spectroscopy, in magnet exercise. Software capabilities will include automatic shimming, automatic phase optimization, and accurate real-time phase calculations. By January 1986, we project sodium and phosphorus imaging with specialized coil design and construction. Specialized isolettes will be constructed for specific experiments (i.e., in-magnet exercise, heart study, brain study).

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The resource provides the necessary instrumentation, technical expertise, instruction, advice, and collaborative opportunities to clinicians and biomedical researchers for in vivo monitoring of specific metabolites in localized regions of tissues and organs in humans and large animals, utilizing multinuclear NMR spectroscopy and metabolic imaging.
- *Current Research:* Evaluation of the several nuclei available for study of brain biochemistry in both spectroscopy and imaging for their effectiveness in quantifying landmarks in the pathway from normalcy to cell death caused by hypoxia in brain and heart; determination of mitochondrial capabilities that can be measured in vivo by ^{31}P NMR in terms of PCr/P_i and/or phosphorylation ratio. Reports of current research in this laboratory and other NMR laboratories are documented in our quarterly newsletter, *News of Metabolic Research.*
- *New Concept/Capability:*
—*Technical:* Cryogenic probe design to improve signal-to-noise ratio; improvement of the preliminary design of the "barrel coil" and other resonant structures; development of multiple-tuned probes to facilitate simultaneous acquisition of multinuclear NMR spectra; establishment of automated protocols for collection and analysis of chemical shift images; development of protocol for automatic shimming; design of special surface coils for high degree of localization and for global measurements.
—*Biochemical:* Potential users and collaborators will investigate a variety of medical problems using NMR spectroscopy in vivo, including the study of the biophysical and metabolic parameters in cancerous and normal tissue; the pathway of liver damage from fatty liver to cirrhotic liver; myocardial metabolism and function at various stages of volume overload hypertrophy; mitochondrial energy metabolism and oxygen delivery in peripheral vascular disease; effects of benign versus malignant epilepsy on cerebral metabolism; metabolic alterations following acute stroke; energy metabolism of brain in coma and during recovery.

[1]Eleff, S., Kennaway, N. G., Buist, N. R. M., Bank, W. J., and Chance, B., ^{31}P NMR study of improvement in oxidative phosphorylation by vitamins K_3 and C in a patient with a defect in electron transport at complex III in skeletal

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

John Haselgrove, Ph.D.
(Software Systems Manager)
(215) 898-5090
Hari Subramanian, Ph.D.
(Operations Manager)
(215) 898-1052

Services Available

- *Service Personnel:* Facility personnel operate the instruments for users; training is provided in operation, theory, curve fitting, and data interpretation.
- *User Laboratory Space:* Laboratory space will be available for animal preparation. A separate area will provide a waiting room and changing room for patients. Computer facility will be available for data analysis in January 1986.
- *User Contact Person:* Michele Locker (215) 898-4342; Alan McLaughlin (215) 898-8033.

Research Emphasis or Application

muscle. *Proceedings of the National Academy of Sciences* 81:3529-3533, 1984.
[2]Younkin, D. P., Delivoria-Papadopoulos, M., Leonard, J. C., Subramanian, H. V., Eleff, S., Leigh, J. S., Jr., and Chance, B., Unique aspects of human newborn cerebral metabolism evaluated with ^{31}P NMR spectroscopy. *Annals of Neurology.* In press.
[3]Hilberman, M., Subramanian, H. V., Haselgrove, J., Cone, J., Egan, J., Gyulai, L., and Chance, B., In vivo time resolved brain phosphorous nuclear magnetic resonance. *Journal of Cerebral Blood Flow and Metabolism.* In press.
[4]Chance, B., Graham, T., Maris, J., and Leigh, J. S., Jr., Resting (state 4) to active (state 3) transitions as observed by ^{31}P NMR in steady state skeletal muscle exercise in normal human subjects. *McMaster International Symposium on Human Muscle Power: Factors Underlying Maximal Performance.* In press.
[5]Nioka, S., Subramanian, H. V., Leigh, J. S., Jr., Forster, R., and Chance, B., Tissue pH regulatory mechanisms in brain in vivo as studied by ^{31}P NMR. *Federation Proceedings* 43:316, 1984.

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

Resource:
Resource for Multinuclei NMR and Data Processing

Principal Investigator:
George C. Levy, Ph.D.
Department of Chemistry
Syracuse University
Syracuse, New York 13210
(315) 423-4026

Services Available

- *NMR Spectrometers:* (1) Bruker WM-360 (8.5 T) wide-bore spectrometer capable of observing all nuclei except ^{19}F and ^{203}Tl. Broadband probes include high-sensitivity side-spinning probes for sample volumes as low as 0.8 ml. Probes also exist for metabolic studies of perfused organs and in vivo experiments on small animals; (2) MOHAWK-250 (5.9 T) standard-bore, broadband spectrometer capable of observing all nuclei; and (3) SENECA-150 (3.5 T) wide-bore, broadband spectrometer capable of observing nuclei in liquid- or solid-state CP/MAS experiments. Side-spinning probes are available for ^{13}C and other nuclei NMR. In 1985, it is expected that a 11.7 Tesla (500 MHz) spectrometer will come online. This resource also has access to a 2.0 Tesla 32-cm bore GE NMR Chemical Shift Imager that will be installed (in 1985) in a shared facility with the SUNY Upstate Medical Center.
- *Computer Hardware and Software:* (1) Data General MV/8000 32 bit superminicomputer. This computer includes 5 Mbytes of memory, 880 Mbytes online disk, four high-speed Lexidata and Raster Technology color graphics systems, approximately 30 serial terminals, and 1200/300 baud modems for remote access. (2) Data General MV/4000 32 bit superminicomputer. This computer includes 4 Mbytes of memory, 700 Mbytes online disk, an ultrahigh-resolution Raster Technology Color Graphics System, and an integrated 32 bit array proces-

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource provides high-resolution NMR spectroscopy for nuclei at several magnetic field strengths. A wide variety of experimental techniques is available to the user. The NMR1 and NMR2 software packages provide powerful reduction of spectral data. These systems allow the extraction of information that is inaccessible with other software packages (quantitative integrals,[1] spectral deconvolutions,[2] extensive regression analyses, and detailed spectral characterization). Information about the molecular dynamics of chemical systems can be obtained by processing multifield NMR experimental data (T_1, T_2, and NOE) with the MOLDYN software package, written at the resource.
- *Current Research:* Structure and conformation of DNA and RNA oligonucleotides; conformational dynamics of single- and double-stranded DNA; ^{31}P metabolism of perfused and in vivo organs from small animals; theoretical modeling of bicpolymer dynamics; studies of enzyme-substrate complexes using transfer NOE and ^{13}C NMR methods; development of sophisticated software systems for spectroscopic data analysis, including approaches to expert systems for in vivo characterization of tissues from magnetic resonance imaging and spectroscopy; high-accuracy quantitative analysis; development of new techniques and software for 2D FT NMR.
- *New Concept/Capability:* Application of resource superminicomputers to high-resolution NMR spectroscopy has enabled the creation of software systems with unprecedented abilities to extract information from one- and two-dimensional data. Algorithms based on rigorous statistical methods allow accurate integration of spectral components having dynamic ranges in excess of 5,000:1 and signal-to-noise ratios as low as 3. Overlapping resonances are easily quantified by a deconvolution module designed to give accurate peak locations, intensities, integrals, and linewidths. Automated processing for complex kinetics, T_1, and other experiments is also fully supported, including the capability to statistically fit equations in up to eight variables (the user types in the equation online). Users may access these capabilities via remote graphics terminals

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Services Available

sor. (3) A VAX 11/750 is on loan to the resource from Digital Equipment to support laboratories wanting to run NMR1 or NMR2 software on VAX computers. Peripherals on most of the above computers include digital plotters, line printers, nine-track tape drives (800/1600 BPI), and word processing printers. These computers provide FORTRAN 77, PASCAL, and C support under Data General AOS/VS and UNIX operating systems. The Data General Computers are networked using Xodiac; also, they may be accessed on CS/NET.

- *Special Features:* Advanced high-resolution NMR techniques such as two-dimensional FT, transfer NOE, cross-polarization, spectral editing, T_1 and exotic decoupling experiments are available. Experiments exceeding 60 hours are possible. In-house designed software for one- and two-dimensional spectroscopy (NMR1 and NMR2) permits the maximum extraction of information from experimental data. Instrument customization is possible for creating new experiments for users in collaboration with the resource staff. Software customization is also available.
- *Service Personnel:* Resource staff include NMR spectrometer operators, biochemists, scientific programmers, and instrument designers. Collaboration with staff members is encouraged. Frequent spectrometer users are trained to run the instruments on request.

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Research Emphasis or Application

(standard phone link). Multifrequency ^{13}C and ^{31}P NMR measurements are performed on solutions of biopolymers. This information is utilized in an advanced software package, MOLDYN[3], to determine the characteristics of overall molecular and internal motional dynamics under various conditions. In 1985, magnetic resonance imaging studies will be initiated. Other NMR specialties include studies of enzyme-substrate complexes using ^{1}H transfer NOE measurements and isotopic labeling with ^{13}C spin-relaxation experiments. The laboratory has developed unique software capabilities for doing quantitative analysis by NMR, particularly for difficult multinuclei bio NMR spectra.

[1]Sotak, C. H., Dumoulin, C. L., and Levy, G. C., *Analytical Chemistry* 55:782-787, 1983.
[2]Kumar, A., Sotak, C. H., Dumoulin, C. L., and Levy, G. C., *Computer Enhanced Spectroscopy* 1:107-115, 1983.
[3]Craik, D. J., Kumar, A., and Levy, G. C., *Journal of Chemical Information and Computer Sciences* 23:30-38, 1983.

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Services Available

- *User Laboratory Space:* The resource biochemistry laboratory has a 4°C cold room, slab gel electrophoresis equipment, and standard biochemical instrumentation. A wet laboratory is also available for organic preparations. Office space is provided for extended visits. Computer graphics terminals are available for users for local or remote (via phone link) processing.
- *User Contact Persons:* Philip N. Borer, Ph.D. (315) 423-1021; George C. Levy, Ph.D., (315) 423-4026.

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

Resource:
Stanford Magnetic Resonance Laboratory

Principal Investigator:
Oleg Jardetzky, M.D., Ph.D.
Stanford Magnetic Resonance Laboratory
Stanford University
Stanford, California 94305
(415) 497-6153

Services Available

- *Instruments:* JEOL GX-500 ^{1}H, ^{13}C cryospectrometer with scheduled replacement of presant magnet with a 600 MHz Tesla magnet. Modified Bruker HXS-360 high-resolution (360 MHz) NMR crycspectrometer system ^{1}H, ^{13}C, ^{19}F, ^{31}P; Nicolet 1180 computer.
- *Special Features:* Plexue software using DEC PDP-11/23 computer system; many spectrometer functions, including sample handling, are under computer control. Software for FT, Redfield, and correlation spectroscopy; continuous, time-share, and pulsed modes available; tau value cycling for relaxation studies; quadrature detection; relaxation times; and 2 DFT.
- *Service Personnel:* Resource staff available on limited basis to provide assistance. All long-term and some short-term users must provide own operators who require minimal assistance. Training, consultation, data reduction available. Collaboration with staff members is encouraged.
- *User Laboratory Space:* Limited space available for preparation of simple samples. Resource supplies all materials related to the instrument. User supplies all materials related to sample preparation. Basic fee charged for instrument time and supplies. Short-term user requests are reviewed and scheduled monthly. Long-term requests are reviewed semiannually.
- *User Contact Person:* Robin Holbrook, (415) 497-6270.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource provides high-resolution NMR spectroscopy at a high-field strength for the study of biological macromolecules in vitro and in vivo. Changes in chemical shift and relaxation rates allow the study of conformational changes and molecular mobility. Classes of problems include binding of substrates, denaturation and renaturation, distinction of flexible regions, and detection of internal motions of macromolecules, and metabolic control in vivo.
- *Current Research:* Macromolecular structure and dynamics: Problems of protein folding, conformational transitions in macromolecules, macromolecular binding sites, and the behavior of living membranes. Metabolism and metabolic control in vivo; nucleic acid dynamics; CIDNP in models for the electron transfer reaction in photosynthesis; lipid-protein interactions and lipid dynamics in native retinal rod and sarcoplasmic reticulum membranes; structural studies of nucleic acids and nucleotides; marine chemistry with special emphasis on steroids; constituents of toxic marine organisms; and structure and function of peptide hormones.
- *New Concept/Capability:* Extensive studies of protein and nucleic acid dynamics have led to a new theoretical formulation of the analysis of relaxation data on macromolecules and a conception of protein dynamics incorporating solition theory. Application of ^{1}H NMR to provide detailed characterization of the mechanism of the interaction of the trp-repressor with effectors and with DNA. Application of ^{31}P, ^{13}C, and ^{1}H NMR to study plant/animal metabolism, and the development of flow systems suitable for long-term perfusion of cells and tissues.

[1]Gonzalez-Mendez, R., Wemmer, D., Hahn, G., Wade-Jardetzky, N. G., and Jardetzky, O., *Biochemica and Biophysica Acta Reviews* 720:274, 1982.
[2]Ribeiro, A. A., King, R., and Jardetzky, O., *Conformation in Biology*, New York, N.Y., Adenine Press, Vol. 39, 1982.
[3]Walpoth, B., Bless, N., Modry, D., Jardetzky, N., Jardetzky, O., Jamieson, S., and Shumway, N., *Heart Transplantation* 2:273-277, 1983.
[4]Jardetzky, O., *Frontiers of Bioorganic Chemistry and Molecular Biology*, Elsevier, 1984.
[5]Ribeiro, A. A., Parello, J., and Jardetzky, O., *Progress in Biophysics and Molecular Biology* 43, 1984.
[6]Roberts, J. K. M., and Jardetzky, O., *New Comprehensive Biochemistry*, Elsevier Biomedical Press. In press, 1984.

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Biological Structure and Function: Nuclear Magnetic Resonance

Title of Resource and Principal Investigator

Resource:
University of California, San Francisco, Nuclear Magnetic Resonance Laboratory

Principal Investigator:
I. D. Kuntz, Ph.D.
University of California
Department of Pharmaceutical Chemistry
Room 926-Science
San Francisco, California 94143
(415) 666-1937/1225

Coinvestigators:
T. L. James, Ph.D.
(415) 666-1569
N. O. Oppenheimer
(415) 666-3038

Services Available

- *Instruments:* GE/Nicolet Proton NMR Spectrometer operating at 500 MHz. Digital Equipment Corporation VAX 11-750 dedicated computer.
- *Special Features:* High-sensitivity, excellent line-shape instrument used primarily for 2-dimensional NMR of biopolymers. 16 bit a/d converter. Distance Geometry Software for data analysis. Ethernet link to UCSF Computer Graphics Laboratory.
- *Service Personnel:* V. Basus, facility manager; M. Moseley.
- *User Laboratory Space:* Instrument space only for spectrometer, computer, terminals, console. Sample preparation must be done in satellite laboratories.
- *User Contact Person:* I. D. Kuntz, (415) 666-1937/1225; V. Basus, (415) 666-3027.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* High-quality NOESY/COSY experiments with excellent water suppression. Software to analyze data—providing 3-dimensional solution structure with sufficient data.
- *Current Research:* See publications.
- *New Concept/Capability:* Quantitative determination of molecular structure in noncrystalline environments using nuclear Overhauser effects and spin labeling. Data are converted to structure using distance geometry calculations and computer graphics.

[1]Havel, T. F., Kuntz, I. D., Crippen, G. M., Theory and practice of distance geometry. *Bulletin of Mathematics and Biology* 45:665-720, 1983.
[2]Schmidt, P. G. and Kuntz, I. D., Distance measurements in spin-labeled lysozyme. *Biochemistry* 23:4261-4266, 1984.
[3]Rosevear, I. P., Sellin, S., Mannervik, B., Kuntz, I. D., and Mildvan, A. S., NMR and computer modeling studies of glutathione derivatives at the active site of glyoxalase. *Journal of Biological Chemistry,* In press.

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Biological Structure and Function: X-ray Absorption and Diffraction

Title of Resource and Principal Investigator

Resource:
National Biostructures Participating Research Team

Principal Investigator:
Britton Chance, Ph.D.
University City Science Center
Institute for Structural and Functional Studies (ISFS)
3401 Market Street #320
Philadelphia, Pennsylvania 19104
(215) 898-4342, 386-1912

Services Available

• *Instruments:* Beam line X9 at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL). Two lines will be built, one specialized for EXAFS (Extended X-ray Absorption Fine Structure) and Edge studies and the other for diffraction and scattering studies. Efforts are being concentrated on building a high photon-flux unfocused beam of 15 milliradians at the end station 6 x 200 mm. This development is especially for the study of dilute metalloenzymes and proteins. This system, which is expected to be in operation early in 1985, will be available for experimental studies. Second phase is a monochromator modification to include a sagittally focusing crystal and vertically focusing mirror by June 1985. The change from unfocused to focused operation is relatively simple. The following developments are relevant to the Edge, XANES, and EXAFS studies:

—Scintillation detectors and integrating detectors are available together with large area x-ray filters and associated electronics for operating either in the pulse counting or the integrating mode.

—Appropriate cryostats are available for fluorescence EXAFS measurements at −100° C and an He cryostat is available for studies down to 4 K.

—A carrier modulation system for separately detecting amplitude and position noise of the beam is available and can be used either in the focused or unfocused mode.[1]

—An optical pumping system is available for stabilizing in time intermediate states of photo-

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Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* EXAFS and XANES provide information about the local structure around selected elements in solids, liquids, or molecular gases. At present, the accessible elements have atomic numbers Z with $19 \leq Z \leq 32$ and $46 \leq Z \leq 72$, e.g. Fe in myoglobin. Using EXAFS, one precisely determines nearest neighbor bond lengths; other structural information such as coordination numbers, atomic number of neighboring atoms, disorder factors, and distances to non-nearest neighbor atoms (out to ~ 4 A) is obtainable with lower precision. XANES spectra are obtained in the same manner as EXAFS and provide some limited information about the nearest neighbor bond angles (e.g., distinguish between tetrahedrally and octahedrally coordinated Fe), as well as approximate bond lengths and valence states (with appropriate model compounds). Low temperature (liquid N_2 or He) measurements are routine, as are measurements of ~ 1-5 mM metalloprotein solutions. Resonance (or anomalous) and nonresonance x-ray scattering/diffraction data can be utilized in both time-averaged and time-resolved structural studies of proteins in solution, partially ordered arrays and perfect crystals to ordered arrays and perfect crystals to provide structural information on length-scales of $1A\text{-}10^4$ A.

• *Current Research:* Edge and EXAFS studies of distance parameters in low-temperature nuclear tunnelling processes in carboxymyoglobin,[2] active site structures in functional activity of cytochrome oxidase,[3] peroxidase, alcohol dehydrogenase, stellacyanin, carbonic anhydrase, and many other oxido-reduction enzymes, either soluble or membrane bound. Research in scattering/diffraction currently concerns the use of both nonresonance and resonance x-ray scattering/diffraction to provide key information concerning the time-average intramolecular structure and intermolecular ordering of membrane molecular components within partially ordered arrays of isolated or reconstituted membranes. Time-resolved scattering/diffraction studies currently center on the investigation of larger-scale structural changes associated with the biological function of certain membrane proteins.[4] Research in protein crystallography centers on

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Services Available

lyzable compounds, for example, hemoprotein-CO compounds. This system is available either for low temperature or room temperature studies.

—A continuous (regenerative) flow apparatus is available for time-resolved reactions in the millisecond region. X-ray observation chambers that increase the detection sensitivity and increase the fluid sample economy are under development.

The following developments are relevant to the scattering/diffraction studies:

—A silicon-intensified target (SIT) detector is under development as an area x-ray detector for diffraction and scattering studies. The detector is controlled by a PDP-11/23 computer.

—A Huber 4-circle diffractometer will be installed. It will be modified to be outfitted with a SIT area detector. The diffractometer is controlled by a PDP-11/23 via a CAMAC interface. It will provide general data acquisition capabilities for diffraction and scattering, including protein crystallography.

Also available are the following:

—A Jarrell-Ash fixed anode x-ray generator is available for sample characterization.

—Core Computer: VAX/VMS 11/730 (2MB memory, floating point accelerator) R80 120 MB fixed disk, RL02 removable disk, TS11 tape drive, Tektronix graphics terminals, HP plotter, line printer. ISFS staff are active in software development. Programs for EXAFS analysis, theoretical calculation of EXAFS/XANES spectra and molecular orbitals, simulation of enzyme kinetics, and scattering/diffraction data reduction.

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Research Emphasis or Application

the use of synchrotron anomalous dispersion methods of phasing. Recent work on the crystal structure of *Pseudomonas dentrificans* of azurin has resulted in the determination of the Cu stereochemistry by use of Cu anomalous dispersion phases bolstered by the Cu partial structure contribution.[5] Other current research includes: experimental and theoretical studies of x-ray absorption processes in condensed matter; interrelation between multiple and single scattering theories of x-ray absorption; EXAFS of disordered systems; improved methods of EXAFS and XANES data analysis; structure/electron transfer (ET) relations in Fe-S proteins and cytochromes; electron-photon interactions in ET.

- *New Concept/Capability:* The use of the high-intensity x-ray radiation and its time-structure from an electron storage ring dedicated for this purpose will give access to dynamic EXAFS studies on the function of metalloproteins as well as time-resolved scattering/diffraction studies concerning the function of membranes, muscle, etc. Special instrumentation for small-signal, high precision data acquisition is featured.

[1]A time resolved modulated-carrier rapid flow system for following rapid structural changes in biological reactions. *Nuclear Instrumentation and Methods in Physics Research* 222:180-184, 1984.

[2]Chance, B., Fischetti, R., and Powers, L., The structure and kinetics of the photoproduct of carboxymyoglobin at low temperatures, an x-ray absorption study. *Biochemistry* 22:3820-3829, 1983.

[3]Powers, L., Chance, B., Ching, Y., and Angiolillo, P., Structural features and the reaction mechanisms of cytochrome oxidase: Iron and copper x-ray absorption fine structure. *Biophysics Journal* 34:465-498, 1981.

[4]Blasie, J. K., Herbette, L., Pierce, D., Pascolini, D., Scarpa, A., and Fleischer, S., Static and time-resolved structural studies of the Ca^{++}–ATPase of isolated sarcoplasmic reticulum. *International Conference on Transport ATPases, Annals of the New York Academy of Sciences*, New York, 402:478-484, 1983.

[5]Korszun, Z. R., and Bolin, J. T., Progress toward the tertiary structure of *Pseudomonas dentrificans* azurin: Synchrotron anomalous dispersion phasing. *Proceedings of the Conference on New Methods in X-ray Absorption Scattering and Diffraction for Applications in Structural Biology*, Academic Press (In press).

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Biological Structure and Function: X-ray Absorption and Diffraction

Services Available

• *Special Features:* This joint resource provides essential facilities and innovative technical developments for EXAFS/XANES and scattering/diffraction studies of biological material. The PRT wishes to facilitate the study of model systems by x-ray spectroscopic and scattering techniques. Such studies should strengthen the fundamental physical-chemical understanding of matter itself and/or of the x-ray techniques utilized. The Biostructures PRT maintains technology at the front line of synchrotron-related developments for biochemists and biophysicists. Staff is available for assistance, collaboration, and training of new users. Beam intensity noise modulation of synchrotron orbital radiation as obtained from bending and wiggler magnets has been analyzed by fast-Fourier transform methods and has identified the principal frequencies of noise intensity modulation at the Stanford Synchrotron Radiation Laboratory (SSRL), CHESS, and DESY. In addition, a special study of beam position noise modulation has been made at DESY. These results are available in various reports. Carrier modulation frequency systems at 10-100 Hz are available for precision EXAFS measurements. A training program for potential users is currently under way, including hands-on experience, lectures, sample preparation, data acquisition and analysis, and equipment preparation.

• *Service Personnel:* Resource staff includes Gerd Rosenbaum, director of beam line con-

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Services Available

struction; three other Ph.D. staff scientists in chemistry and physics; instrument designers, scientific and technical programmers, and engineers. Members of the Executive Committee are available for technical and scientific advice. Staff members are experienced in EXAFS/XANES and x-ray scattering/diffraction and are available for consultation and training.

- *User Laboratory Space:* The resource is currently performing experiments at SSRL and CHESS at Cornell until the beam line at the NSLS/BNL synchrotron source is completed in 1985. The core laboratory, shop, x-ray generator facility, computer core (LSI 11/23 and VAX 11/730), and offices have been established at the Institute for Structural and Functional Studies of the University City Science Center in Philadelphia. Once the beam line at NSLS is complete, significant operations will be conducted at Brookhaven as well.
- *User Contact Persons:* Britton Chance, Ph.D., (215) 898-4342; Gerd Rosenbaum, Ph.D.; Z. Richard Korszun, Ph.D.; Grant Bunker, Ph.D.; and Syed Khalid, Ph.D., in Philadelphia, (215) 386-1912; Mike Sullivan, on-site at BNL/NSLS beam line X9, (516) 282-5609.

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Biological Structure and Function: X-ray Absorption and Diffraction

Title of Resource and Principal Investigator

Resource:
Synchrotron Radiation Biomedical Research Technology Resource

Principal Investigators:
Arthur I. Bienenstock, Ph.D.
Director
Stanford Synchrotron Radiation Laboratory
Bin 69, SLAC
Stanford, California 94305
(415) 854-3300 x3153

Keith O. Hodgson, Ph.D.
Associate Director
SSRL
Department of Chemistry
Stanford University
Stanford, California 94305
(415) 497-1328

Services Available

• *Facilities:* The Synchrotron Radiation Biomedical Research Technology Resource is located at the Stanford Synchrotron Radiation Laboratory (SSRL). The laboratory is a national facility supported primarily through the U.S. Department of Energy for the research utilization of synchrotron radiation. A biochemical/chemical laboratory has been constructed and equipped at SSRL. A computational support facility houses a digital VAX-11/780 computer system to support scientific development in the four research areas of the SSRL Biomedical Research Technology Resource and to enable users to assess the quality of data through some analysis while still on site.

• *Instruments:* The SSRL is uniquely equipped for spectroscopic and scattering studies spanning the spectrum from X ray into the infrared. The new biochemical laboratory houses an ultracentrifuge, scanning UV-visible spectrophotometer, milligram balance, and stereomicroscope. Support facilities include a millipore deionized water system, icemaker, refrigerator-freezer, laboratory glassware washer, and a large cold box for chromatography. Other routine equipment and supplies are also provided. A digital VAX-11/780 computer system was installed in 1980.

• *User Contact Person:* K. Cantwell, assistant to the director, SSRL, (415) 854-3300 x3191.

Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* X-ray absorption spectra using absorption and fluorescence detection; x-ray diffraction data on single crystals and oriented membrane samples with variable wavelength; and fluorescence lifetime measurements on materials in the nanosecond region.

• *Current Research:*
—*Protein Crystallography:* The grant provides support for the development of the SSRL area detector system. This multiwire chamber area detector system is now functional. Programs for data reduction are being implemented on the VAX-11/780 so that users will be able to leave the site with their data already reduced. Two research associates are working on the development of the area detector and associated software to develop techniques in protein crystallography using synchrotron radiation for phasing based on anomalous scattering. Wavelengths of about 0.5-2 A are available for protein crystallographic studies. A new rotation camera beam line has been installed on a wiggler source (Beam Line VII-1) at SSRL. This station provides the means for users to collect protein rotation data sets over a large range of wavelengths. Photon flux is comparable to or higher than similar facilities available in Europe. This results in very short exposure times and in many cases will enhance the resolution obtained in comparison with conventional sources. It should also be possible to measure data from smaller crystals.

—*Small-Angle Scattering:* A resource for small-angle scattering from biological materials is being supported in this research area. A general purpose, end-station hutch can be used on the end stations of BL II, IV, or VII. A small-angle scattering camera has also been constructed. The camera incorporates a one-dimensional position-sensitive wire detector. The output of the detector is monitored by a multichannel analyzer from which data can be transferred in turn directly to any computer at SSRL, via a CAMAC crate, for storage on disk.

—*EXAFS:* Fluorescence detectors that permit measurement of x-ray absorption spectra from millimolar concentrations of biological systems have been acquired. Further development of the fluorescence detection systems that are necessary for EXAFS studies of more dilute biological systems is being carried on. Energies accessible at SSRL for EXAFS studies

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Research Emphasis or Application

span about 4-30 keV. A set of interactive programs has been implemented that will allow users to analyze EXAFS data and display the results on-line.

—*Nanosecond Fluorescence Studies:* Instrumentation has been set up to provide the capability of carrying out fluorescence lifetime studies in the "hutch" on the top of the SPEAR ring. A time resolution of at least 100 ps is currently available. The apparatus consists of monochromator and single photon counting electronics. Direct data transfer from the MCA to disk storage is now possible.

- *New Concept/Capability:* This resource is designed to enhance capabilities for biologically related research in protein crystallography, small-angle scattering, further development of fluorescence detection systems, and nanosecond fluorescence studies. Further details of SSRL and its operations and programs can be found in the annual SSRL activity report available by writing to the laboratory.

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Biological Structure and Function: X-ray Diffraction

Title of Resource and Principal Investigator

Resource:
Macromolecular Diffraction Resource, MacCHESS

Principal Investigator:
Keith Moffatt, Ph.D.
Section of Biochemistry, Molecular and Cell Biology
Clark Hall
Cornell University
Ithaca, New York 14853
(607) 256-4677

Coinvestigators:
Boris Batterman, Ph.D.
(607) 256-5161
Donald Bilderback, Ph.D.
(607) 256-7163

Services Available

- *Instruments:* Monochromators on the three diffraction beam lines deliver either focused nontunable radiation at around 1.57 A, focusing tunable radiation in the range from 0.7 to 2.0 A, or nonfocusing tunable radiation down to 0.3 A. Diffraction apparatus includes a Huber 4-circle diffractometer, a Nonius oscillation camera, and a super precession-oscillation camera. A crystal cooler that covers the range from −50 to +70 C° is available.
- *Facilities:* MacCHESS is located at the Cornell High-Energy Synchrotron Source, CHESS. CHESS is a national laboratory supported by the National Science Foundation for research that employs synchrotron radiation. CHESS has three beam lines that currently serve six experimental stations. Three of the stations are suitable for diffraction experiments using monochromatic radiation, two for EXAFS or XANES experiments, and one for experiments that utilize the white synchrotron beam. The first three are served by a six-pole wiggler magnet and the remainder by bending magnets.
- *Service Personnel:* A CHESS operator is available at all times. MacCHESS personnel assist in aligning the diffraction apparatus and instructing experimenters in its use.
- *User Laboratory Space:* A support laboratory is available for crystal mounting and apparatus setup, together with a small darkroom, machine shop, and limited computing facilities.
- *User Contact Person:* Keith Moffat, Ph.D., (607) 256-4677; Boris Batterman, Ph.D., (607) 256-5161.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Diffraction data from single crystals and oriented fiber or membrane samples with monochromatic or polychromatic tunable radiation.
- *Current Research:* The development of novel x-ray optics and diffraction techniques. Optics under investigation include focusing mirrors, transmission mirrors, and layered synthetic microstructures, suitable for both monochromatic and polychromatic diffraction. Most outside users have employed the focusing nontunable radiation and an oscillation camera. Unit cell dimensions up to 500 A have been readily resolved (and larger dimensions up to 1000 A could probably be resolved also). Typical exposure times per photograph are 2 to 5 minutes. Crystals with a volume as low as 10^{-5} cubic microns have been examined readily.
- *New Concept/Capability:* The prospects for Laue diffraction using highly polychromatic radiation are being examined. The long-term goal is the development of time-resolved crystallography, with single-shot, x-ray exposures in the millisecond time range.

[1]Moffat, K., et al., *Science* 223:1423, 1984.
[2]Bilderback, D., et al., *Nuclear Instrumentation Methods* 222:245, 1984.

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Biological Structure and Function: X-ray Diffraction

Title of Resource and Principal Investigator

Resource:
Multiwire Area X-ray Diffractometer for Protein Crystallography

Principal Investigators:
Stanley E. Sobottka, Ph.D.
Department of Physics
University of Virginia
Charlottesville, Virginia 22901
(804) 924-6802

Robert H. Kretsinger, Ph.D.
Department of Biology
University of Virginia
Charlottesville, Virginia 22901
(804) 924-7039

Services Available

- *Instruments:* Multiwire area diffractometer, three-circle goniostat, and sealed-tube x-ray source for measuring x-ray diffraction intensities from protein crystals; software and HP 1000/45 computer for instrument control and data reduction to supply user with Lorentz-polarization corrected intensities F^2_{hkl}.
- *Service Personnel:* An operator is available at all times while an external user is in residence.
- *User Laboratory Space:* Available for crystal mounting and optical orientation.
- *User Contact Person:* Robert H. Kretsinger, Ph.D., (804) 924-7039.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Measurement of diffracted x-ray intensities. Unit cell resolution, 180 A. X-ray photon counting rates, 10^5 to 10^6 cps per detector. Large protein crystals (0.4 mm on a side) diffract 10^4 to 10^5 cps with our Cu, sealed tube fine-focus source operated at 40 kV, 25 milliamperes. Data collection times are reduced over a factor of 10, compared with present single-counter diffractometers or oscillation film methods.
- *Current Research:* Our present research involves designing, constructing, and testing this area diffractometer, and has already led to improved delay line designs and better time-digitizing devices.
- *New Concept/Capability:* The ability to measure very rapidly accurate diffraction data not only facilitates standard protein structure determination but also allows new sorts of experiments: Use of smaller and/or radiation-sensitive crystals, more effective use of anomalous dispersion data, examination of extensive series of modified structures, and kinetic measurements.

[1]Sobottka, S. E., Cornick, G. G., and Kretsinger, R. H., in *Workshop on X-ray Instrumentation for Synchrotron Radiation Research* (Winick, H., and Brown, G., eds.). 8:70, 1978.
[2]Sobottka, S. E., Cornick, G. G., Kretsinger, R. H., Rains, R. G., Stephans, W. A., and Weissman, L. J., A MWPC x-ray diffractometer facility for protein crystallography. *Nuclear Instruments and Methods in Physics Research* 220:575-581, 1984.

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Biological Structure and Function: X-ray Diffraction

Title of Resource and Principal Investigator

Resource:
Resource for Protein Crystallography

Principal Investigator:
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Department of Physics
University of California at San Diego
La Jolla, California 92093
(619) 452-2501

Coinvestigator:
Ronald Hamlin
(619) 452-2565

Services Available

- *Instruments:* A high-speed data collection system for protein crystallography consisting of a rotating anode x-ray generator, a three-circle goniostat, and two multiwire area detectors. The crystal is oriented by the three-circle goniostat and can be cooled down to −30°C by a dry nitrogen cooling device. A dedicated VAX 11/750 computer not only controls the orientation of the crystal and the gathering of x-ray data from the area detector but also analyzes the data to extract immediately the integrated intensity of the recorded reflections.
- *Service Personnel:* During the first few days, the user will be guided by an in-house protein crystallographer who will assist him during the entire data collection period. However, the system is very easy to learn, and a user will have no trouble running it after the first two days. Once the crystal is mounted and oriented, the data collection is done automatically for days with little intervention by the operator.
- *User Laboratory Space:* Available for crystal mounting and optical orientation.
- *User Contact Person:* Nguyen-huu Xuong, Ph.D., (619) 452-2501.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Excellent measurements of diffracted x-ray intensities from protein crystals with unit cell dimensions as large as 250 A. Due to the high speed of data collection (over one hundred times faster than a standard diffractometer), every reflection intensity will be measured many times from a single crystal. The data quality is excellent, with precision on the order of 5 to 6 percent. During the last year, more than 6 million reflection intensities have been collected in 17 different projects.
- *Current Research:* New methods to collect data with higher speed and precision.
- *New Concept/Capability:* Use of Multiwire Area Detector accelerates by a factor of 100 the data collection rate from protein crystals, therefore allowing the structure determination of very large and interesting enzymes. It also opens the door to the structure determination of relatively unstable enzyme-substrate complexes.

[1]Cork, C., Hamlin, R., Vernon, W., Xuong, N. h., and Perez-Mendez, V., *Acta Crystallography* A31:702-703, 1975.
[2]Xuong, N. h., Freer, S., Hamlin, R., Nielsen, C., and Vernon, W., *Acta Crystallography* A34:289-296, 1978.
[3]Xuong, N. h., Nielsen, C., Hamlin, R., and Anderson, D., *Journal of Applied Crystallography*, In press.

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Biological Structure and Function: X-ray Microprobe

Title of Resource and Principal Investigator

Resource:
National Synchrotron Light Source
X-ray Microprobe Facility

Principal Investigator:
Keith W. Jones, Ph.D.
Department of Applied Science
Brookhaven National Laboratory
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(516) 282-5125

Coinvestigators:
Barry M. Gordon, Ph.D.
(516) 282-5125
Albert L. Hanson, Ph.D.
(516) 282-5125

Services Available

- *Instruments:* The X-ray Microprobe Facility is located on beam port X-26 at the Brookhaven National Laboratory National Synchrotron Light Source. The facility is now under construction. When complete, use of monochromatic X rays in the range from 4 to 17 keV or white synchrotron radiation above 4 keV with spatial extent from 30 μm^2 to about 1.6 mm x 8 mm will be used for trace element determinations by the x-ray fluorescence method. Target chambers, sample stages, energy dispersive detectors, and wavelength dispersive detectors are available. In special cases, arrangements can also be made to use ion beams from a 3.5-MV Van de Graaff Accelerator to complement the NSLS x-ray microprobe capability. Ion beam sizes from approximately 2 cm to 20 μm are possible with corresponding beam currents between 50 μA and 25 nA. Nuclear reaction analysis, Rutherford backscattering, resonance depth profiling, and proton induced x-ray emission (PIXE) measurements can be made with these beams. This accelerator provides several techniques that complement and extend the capabilities of the x-ray microprobe device.
- *Special Features:* This facility will provide sensitive measurements of elemental concentrations with sensitivities as low as 10 ppb for measurements on bulk samples. Measurements can be made on wet or thick samples in vacuum or nonvacuum environments. The techniques complement other methods such as

Continued on next page

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The use of the x-ray beams produced by NSLS will make possible determination of elements between Al and U, not only with sensitivities of 100 ppb or better, but also ultimately with spatial resolutions of around 5-10 μm. In a 20-μm thick tissue sample and a spot size of 10 μm, this will correspond to detection of about 10^6 atoms. Great flexibility is possible in the nature of the sample because of the penetrating power of the X rays. Radiation damage from the X rays is in order of magnitude less than for ionizing beams such as electrons for similar detection sensitivities. The attributes of the MeV ion beams are almost as good. Light-element determinations are superior; sensitivities with PIXE are not as good.
- *Current Research:* Current research carried on by the scientists involved with the facility is concerned with basic studies to improve the x-ray fluorescence technique in detection sensitivity and spatial resolution, development of new analytical techniques, and the study of the correlation of cellular and tissue localization of toxic and essential metals with the cell functions mediated by essential metals.
- *New Concept/Capability:* The application of the ultrahigh brightness x-ray source provided by the NSLS to x-ray fluorescence measurements will make possible ultrasensitive, spatially resolved trace element determinations not feasible up to the present time. Complementary ion-beam methods are only now coming into widespread use. Taken together, the capabilities at the facility make possible many new experiments.

[1]Gordon, B. M., Uses of synchrotron radiation. *Neurotoxicology* 4:13, 1983.
[2]Jones, K. W., Gordon, B. M., Hanson, A. L., Hastings, J. B., Howells, M. R., Kraner, H. W., and Chen, J. R., Application of synchrotron radiation to elemental analysis. *Nuclear Instruments and Methods* B3:225, 1984.
[3]Geisler, F. H., Jones, K. W., Fowler, J. S., Kraner, H. W., Wolf, A. P., Cronkite, E. P., and Slatkin, D. N., Deuterium micromapping of biological samples by using the D(T,n)^{4}He reaction and plastic track detectors. *Science* 186:361, 1974.

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Services Available

inductively coupled plasma emission spectroscopy (ICP), electron and ion microprobes, or mass spectroscopy, which are used for determination of trace elements.

- *Service Personnel:* Facility personnel are available to train and supervise operation of the x-ray microprobe facility by users. It is expected that users will generally be present to actively participate in the data-taking process.
- *User Laboratory Space:* Some facilities will be provided for sample preparation at Brookhaven. Users should try to perform most of their preperation work at their home institution.
- *User Contact Person:* Ms. Lore M. Barbier, (516) 282-5125.

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Title of Resource and Principal Investigator

Resource:
Semiconductor Chemical Transducer Resource

Principal Investigator:
Chung C. Liu, Ph.D.
Electronics Design Center
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Cleveland, Ohio 44106
(216) 368-2934

Coinvestigators:
Peter W. Cheung, Ph.D.
David G. Fleming, Ph.D., M.D.
Clifford D. Fung, Ph.D.
Wen H. Ko, Ph.D.
(216) 368-2934

Services Available

- *Instruments:* The resource has facilities for solid-state electronic devices and sensor design, fabrication, and evaluation specifically in the areas of: single ISFET sensors, electrolyte multiprobe development, ISFET-based blood gas sensors (pH, PCO_2), chemically specific CHEMFET sensors using immobilized enzymes and new sensor structure design and fabrication. Facilities include: full bipolar and MOS integrated circuit facilities, diffusion systems, a 300 keV ion implantation unit, an infrared spectrophotometer, a microphotolithograph, a chemical vapor deposition unit, plasma etching and deposition equipment, various vacuum deposition systems, photomask-making capabilities, CO_2 and Nd:glass lasers, Auger analysis equipment, a scanning electron microscope, and computer-aided design capabilities.
- *Special Features:* Collaborative research and industrial interaction; design and fabrication of specialized chemical transducers and sensors; and training that includes workshops,[1] seminars, predoctoral and postdoctoral traineeships, and a visiting scientists' program.
- *Service Personnel:* A professional team to collaborate with investigators for semiconductor chemical transducer research, development, and applications that include: a chemical transducer and electronics laboratory working at a high technology level on theory, new structures, fabrication, technology, packaging, and biomedical applications of semiconductor chemical sensors including training and dissemination of technical information.

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource provides a specialized facility for applying modern microelectronics technology to the development of semiconductor chemical transducers. A generic class of miniature semiconductor chemical sensors[2] for pH and PCO_2 determination and electrolyte measurements, such as K^+ and Ca^{++} ions for research and clinical applications, is available.
- *Current Research:* Single ISFET sensor research and development,[3] electrolyte multiprobe development, chemically specific CHEMFET sensors using immobilized enzymes, new sensor design and fabrication, integrated circuit multiple transducers, chemical sensing transducer integrated with active circuits, biotelemetry systems, sensor packaging materials and techniques,[4] pH and PCO_2 sensors, and computer-aided transducer design and technology development for transducer fabrication.
- *New Concept/Capability:* Application of micromachining for chemical measurement systems.

[1]Cheung, P. W., Neuman, M. R., Fleming, D. G., and Ko, W. H., *Theory, Design, and Biomedical Applications of Solid-State Chemical Sensors.* West Palm Beach, Fla., CRC Press, Inc., 1978.
[2]Cheung, P. W., Chemical sensors. In *Essential Noninvasive Monitoring in Anesthesia* (Gravenstein, J. S., Newbower, R. S., Ream, A. K., and Smith, N. T., eds.). New York, N.Y., Grune and Stratton, Inc., 1980, pp. 183-216.
[3]Ko, W. H., Lee, J. M., Fung, C. D., and Cheung, P. W., An experimental study on the fringe field-effect of V_{DS} on ISFET pH sensors. *Sensors and Actuators* 3:91-99, 1983.
[4]Ko, W. H., and Spear, T. M., Packaging materials and techniques for implantable instruments. *IEEE Engineering in Medicine and Biology: Biomedical Telemetry* 2:24-38, 1983.
[5]Ko, W. H., Physical transducers. *Proceedings of the IEEE Symposium on Biosensors* September 15-17, 1984, 1.
[6]Cheung, P. W., Semiconductor-based chemical sensors and their application in medicine and biology. *Proceedings of the IEEE Symposium on Biosensors* September 15-17, 1984, 13.
[7]Fung, C. D., Tung, C. Y., and Ko, W. H., Enhanced etching of silicon in SF_6 plasma with DC bias. *Extended Abstracts of Electrochemical Society* October 8-12, 1984, 578-579.
[8]*Abstracts from Workshop on Micromachining and Micropackaging* November 7-9, 1984.

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Biomedical Engineering

Services Available

- *User Laboratory Space:* No separate user space is provided unless arrangements are made through a collaborative project for personnel exchanges.
- *Service Fees:* Collaborative project costs are worked out on an individual basis; informed use of resource equipment is according to availability with an actual cost charge, if resource materials are used.
- *User Contact Person:* Peter W. Cheung, Ph.D., (216) 368-2934.

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Title of Resource and Principal Investigator

Resource:
Autoradiographic Image Processing Center

Principal Investigators:
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Philadelphia, Pennsylvania 19104
(215) 898-6230

Services Available

• *Facilities and Hardware:* The Autoradiographic Image Processing (AIPC) is located in Room 507, Commonwealth Building, Drexel University, Philadelphia, Pennsylvania, and is part of the Drexel University Image Processing Center. The present computer hardware includes: A PDP 11/34A CPU with 256 kbytes of memory, a 134-Mbyte Winchester disk drive, a DEC RKO5 removable disk pack and an 800/1600-bpi tape drive. The image processing equipment is based upon the Spatial Data Eyescan Image Processing System (PT-108). The picture memory is 320 by 240 pixels with a resolution of 8 bits (256 gray levels). There is a user-definable color map (3 bytes x 64 deep) such that each color is capable of 256 intensities. The user interfaces include a joystick controlled cursor and a VT-220 terminal to issue commands. The system provides outputs on a CONRAC 19-inch color monitor, the same VT-220 terminal, and a Printronix 300 printer for hard copies of the analyses.

A microcomputer based image processing system is under development.

• *Software:* The operating system on the present PDP-11/34A is RSX-11 using Fortran IV Plus and MACRO-11 as the higher level and assembly languages, respectively. The new low-cost system will use XENIX 3.0 as the operating system and the higher level language will be C.

The software package for image analysis is menu-driven, offering a number of options for both qualitative and quantitative analysis. The

Continued on next page

Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* Qualitative and quantitative analysis of autoradiographs, including pseudocolor enhancement and optical densities, ^{14}C concentrations, and relative optical densities; general image analysis; superposition of histology on autoradiograph for quantitative analysis.

• *Current Research:* Pattern recognition: Research is being conducted on pattern recognition to facilitate analysis of autoradiographs. We are engaged in creating software to remember user-generated outlines of brain areas to be stored in a data base and then applied to different brain sections. The computer will automatically readjust the outlines on consecutive sections with considerable savings of the user's time and effort.

Low-Cost Image Analysis System: The grant provides for the development of a low-cost system; total price for hardware is less than $20,000. This system is based upon the IBM PC-XT, with Datacube Image Processor and Stritek 68000 coprocessor. The low-cost system will be capable of storing images on floppy disks and will have practically double the capabilities of the present PDP-11 based system.

Standardization and Quality Control: Tests are being conducted on the various sources of error in computer-based image analysis, with the goal of minimizing such error. Various experiments are also in progress to standardize brain preparation for sectioning and control of other physical sources of errors.

• *New Concept/Capability:* This resource is designed to promote the use of and enhance the analysis of experiments involving 2-DG and other radioactive tracers. Besides 2-DG, radiolabeled neurotransmitter or receptor binding, grain counting, and other capabilities are, or will be, developed. The low-cost system is being designed with increased resolution, decreased error, and increased speed. Further details concerning both the AIPC and the low-cost system can be obtained from Dr. McEachron.

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Services Available

system provides for an overlay of the histological section (on live video) onto the digitized autoradiograph, which is stored in picture memory. Standards of optical density and ^{14}C concentration are entered prior to the analysis of sections to allow for quantitative analysis. *Qualitative Analysis.* The program provides for a number of options in pseudocolor enhancement and graphics including: RAINBOW, which provides a computer-generated and standardized color map of ^{14}C-2-deoxyglucose (2-DG) uptake across the section; WINDOW, which is a user-defined color mapping system allowing the user to color enhance specific levels of 2-DG uptake; PROFILE, which generates a map of 2-DG uptake along a 1 pixel wide line in the vertical or horizontal direction defined by the user; GRAPHICS, which allows the user to change colors generated by WINDOW to black-and-white for publication or presentation and provides for labeling of the section on the terminal screen. All black-and-white graphics can be printed out in hard copy. *Quantitative Analysis.* The subroutine QUANTITATIVE is used to generate values in gray level, optical density, ^{14}C concentration, and relative optical density. The user defines the area to be analyzed and the computer generates the means and standard deviations automatically and displays the results on the CRT. These data are also printed for later analysis.

- *Service Personnel:* Facility personnel, including a resident neuroscientist, engineer, and

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Services Available

computer programmers, provide support for the use of the Autoradiographic Image Processing Center. A manual is available free of charge, and the neuroscientist, Dr. Donald L. McEachron, is available for consultation about experimental design and analysis using ^{14}C-2-deoxyglucose. In the spring of 1985, a low-cost system will become available so the AIPC will provide: 1) A list of vendors from whom the equipment can be purchased; 2) the entire software package; 3) personal attention and help in starting up the system.

- *User Laboratory Space:* No separate user space is provided but users are encouraged to utilize the image processing system at the facility where support and help are available.

User Contact Person: Dr. Donald L. McEachron, scientific director, (215) 895-2279.

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Title of Resource and Principal Investigator

Resource:
Biological Computer Facility for Image Processing and Displays

Principal Investigator:
Cyrus Levinthal, Ph.D.
1011A Sherman Fairchild Center for the Life Sciences
Department of Biological Sciences
Columbia University
New York, New York 10027
(212) 280-2439

Services Available

• *Hardware:* DEC VAX 11/780; PDP 11/34; Evans and Sutherland Picture System 2 with two interactive vector display terminals; Grinnell color raster display; high-resolution laser film scanner (ANT); three CARTOS (Computer Aided Reconstruction by Tracing of Serial Sections) digitizing and display work stations; 16-mm animation camera; "image combiner" alignment bench for 35-mm filmstrips. One CARTOS terminal is a CODATA MC68000-based microcomputer system with 35-mm film projector and x-y digitizing tablet; it runs UNIX and has stand-alone 3-D reconstruction programs. This system is available for use on-loan by outside users. The second CARTOS terminal is for light microscope 3-D tracing and counting of stained cells, and consists of a CODATA with ITI image processing boards and interfaces to a digitizing tablet, fine focus controls, and stage controls on a Leitz microscope. The third CARTOS system uses an Evans and Sutherland terminal for 3-D reconstruction from a projected film strip. A fourth system is the ANT (Automatic Nerve Tracing) that combines the laser scanner and the Grinnell display with a tablet, and can be used for manual or semiautomatic tracing from 35-mm film, predigitized images such as CT tapes, or live video images.

• *Software:* UNIX version 4.2; CARTOS tracing programs including ACE (Automatic Contour Extractor) for reconstructing cellular, anatomic, or morphologic information from serial sections; Biostruct, a data manager and editor for dis-

Continued on next page

Research Emphasis or Application

Data Achievable with Resource's Methodologies: This resource provides the capability to study the three-dimensional structure of organelles, cells, and large aggregates of cells, especially for neuroanatomic studies. Both manual and automatic tracing systems are available for collecting three-dimensional information from serial-section light or electron micrographs. The manual tracing systems are available for any anatomic work in which structures are visible in serial-section micrographs (or other images); the automatic system is available for exploratory work with high-contrast material. The facility is also being used for exploratory work on the application of three-dimensional reconstruction techniques to problems of clinical pathology using CAT, PET, NMR, and/or ultrasound imaging systems.

This resource also provides display and calculational facilities for the three-dimensional structure of large biomolecules, with emphasis on proteins, their conformations, interactions, and modifications. Three-dimensional graphics are used to visualize the structures, and large-scale computer calculations are used to refine, modify, and predict new structures and structural interactions.

• *Current Research:* Growth and development of the visual system in embryonic and newborn zebrafish[1] and in *Daphnia magna.*[2] Neuroanatomic reconstruction in *Manduca sexta,*[3] leech,[4] and cat cortex.[5] Human brain atlas in conjunction with analysis and display of CAT scan data, and analysis of anatomic variation in brain structure associated with schizophrenia and Alzheimer's disease. A variety of other projects involving vertebrate and invertebrate neuroanatomy and bacterial ultrastructure. A 3-D rat atlas is now running on the system and is to be used for 3-D mapping of neural peptides on a standard rat atlas.

Molecular modeling and structural predictions on binding sites of several naturally occurring antibody molecules as well as ones modified by recombinant DNA techniques. Molecular modeling of carboxypeptidase to study active site structure and conformational changes accompanying inhibitor binding.

• *New Concept/Capability:* An automatic nerve-tracing system has been developed at the facility that makes possible rapid and

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Services Available

playing and manipulating these reconstructions; CON and PAINT, programs for shaded surface displays of such reconstructions; PAKG-GRAF protein modeling program with energy minimization capability; PDP, a package for selective display of proteins and their component parts; SHADE, a program for color display of space-filling molecular models; GPIC, general purpose graphics package; programming languages including C, FORTRAN, PASCAL, and LISP.

- *Service Personnel:* Facility personnel, chiefly involved in system development and collaborative research, can provide support for the use of CARTOS at the facility, with loaner systems, and for biomodeling in general.
- *User Laboratory Space:* No separate user space is provided, but users can work on the CARTOS systems or the molecular manipulation systems, at the facility.
- *User Contact Person:* Ms. Linda Sproviers, research associate, (212) 280-5517/2439.

Research Emphasis or Application

extensive three-dimensional analysis of neuroanatomic material.[6] This system (ANT or ACE) has been extended to utilize image understanding techniques including digital filtering, image segmentation, guided searching, and adaptive programming. We have designed a special purpose digital processor, designated FASTRUN, for high-speed calculations involved in molecular modeling. FASTRUN will operate on any set of *n* objects that interact with each other in three-dimensional space by means of central forces. It will carry out the inner loop of the interaction calculation and produce a list of forces on each object and the total interaction energy of the configuration. In conjunction with a commercial array processor, FASTRUN could carry out minimizations or energy simulations on the model of a protein at about 1,000 times the speed of a VAX-11/780. Preliminary design and engineering cost analyses have been completed; further work is required to produce a prototype. The design and engineering layout have been completed, and the system is now under construction at the Brookhaven National Laboratory. An operating system is expected to be functional in 1986.

[1]Bodick, N., and Levinthal, C., *Proceedings of the National Academy of Sciences* 77:4374-4378, 1980.
[2]Macagno, E. R., *Journal of Neuroscience* 1:945-955, 1981.
[3]Schneiderman, A. M., Hildebrand, J. G., and Jacobs, J. J., *Society of Neuroscience Abstracts*, In press.
[4]DeRiemer, S. A., and Macagno, E. R., *Journal of Neuroscience* 1:650-657, 1981.
[5]Harris, D. M., *Proceedings of the Society of Neuroscience*, In press.
[6]Sobel, I., Levinthal, C., and Macagno, E. R., *Annual Review of Biophysics and Bioengineering* 9:347-362, 1980.

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Title of Resource and Principal Investigator

Resource:
Biomedical Simulation Resource

Principal Investigator:
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Box 3709
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Durham, North Carolina 28710
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Coinvestigators:
Michael L. Hines, Ph.D.
(919) 684-5748
Michael C. Kohn, Ph.D.
(919) 681-3049
John W. Moore, Ph.D.
(919) 684-5748
Dietolf Ramm, Ph.D.
(919) 684-3048
Charles F. Starmer, Ph.D.
(919) 684-6804

Services Available

• *Hardware:* Applied Dynamics International AD-10 digital simulation processor; array processor; VAX-11/750 host processor with 4 Mb memory, 900 Mb of disk storage, 1600 bpi tape, and dual floppy drives; SUN/120 high-resolution monochrome graphics work station with 160 Mb disk and cartridge tape; IBM PC/XT color graphics work stations; Imagen 8/300 laser printer; HP 7220T and 7475A plotters; links to various graphics terminals and other Duke computer systems through a Network Products dataswitch; link to TYMNET data network.

• *Software:* Programming environment: VAX/VMS and EUNICE (similar to Berkeley 4.1BSD UNIX) on the VAX-1/750 host; 4.2BSD UNIX on the SUN/120; PC-DOS on the IBM PC/XT work stations. Languages supported include FORTRAN and C on the VAX; C, FORTRAN, PASCAL, and LISP under UNIX; C, FORTRAN/77, and PASCAL under PC-DOS. Simulation languages: ACSL, CSSL-IV, MPS-10, and SCoP.

Mathematical packages: IMSL library and PROTRANS, SAS, and SMP. Graphics libraries: PLOT-10, SAS-GRAPH, and GRAPHLIB (similar to SIGGRAPHCORE).

• *Service Personnel:* Resource investigators are available for consultation about model construction and application. Users generally must write their own simulation programs, but the resource's programming staff assists users with questions about system access, languages, libraries, and other facilities.

Continued on next page

Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* Facilities are available to construct and exercise mathematical models of any biomedical system, molecular to ecological, small (one or two equations) or large (hundreds or thousands of equations). Simulation languages and other available libraries simplify and speed the construction of simulation programs. Graphic facilities are available to present simulation output in forms comparable with experimental data, and programs are available to fit models to data. Symbolic manipulation of model equations is available with SMP. USA-wide access through TYMNET permits researchers at different laboratories to share simulation programs and data.

• *Current Research:* The resource staff is developing general-purpose software for simulation including numerical and graphics procedures, procedures describing common physical/chemical mechanisms and laboratory instruments, and filters for selecting regions of interest in simulation output. User projects under way span a broad range from molecular mechanisms to whole organ or animal studies. Some sample subjects are: ion transport in nerve and muscle cells; coupling and propagation between heart muscle cells and at the neuromuscular junction; frequency discrimination in the cochlea; distribution of toxic materials in the lung; hormone-receptor binding; ciliary motility.

• *New Concept/Capability:* For the experienced simulator, the resource provides access to high-speed processors for problems requiring large amounts of computation (e.g., solution of 900 or more simultaneous differential equations and large matrices) as well as access to libraries of languages and subroutines to aid in the construction of programs, fitting of parameters to data, and presentation of simulation results in graphic form. For the novice simulator, the resource offers introductory courses on simulation and an easy-to-use software system for constructing interactive models on personal computers. National access to the resource encourages communication between simulators and sharing of programs and data. A periodic newsletter gives researchers the latest information on simulation technology and activity. Symposia are sponsored by the resource to encourage the development of broad, general models spanning several fields.

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Services Available

- *User Laboratory Space:* For on-campus users, a terminal room is provided with graphic terminals, graphic work stations, plotters, and printers. Remote users can access the computer facilities via TYMNET (a local telephone call in most areas) at 1200 or 300 baud. Remote users must provide their own terminal and modem.
- *Service Fees:* No charge to qualified projects (includes consultation, cpu time, disk storage, and data communication).
- *User Contact Person:* David B. Leonard (919) 681-3048, (800) 334-2083 or, in North Carolina, (919) 672-2534.

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Computer Resources

Title of Resource and Principal Investigator

Resource:
BIONET—National Computer Resource for Molecular Biology

Principal Investigator:
Dr. Eugene Kromer, President
IntelliCorp, Inc.
707 Laurel Street
Menlo Park, California 94025-3445
(415) 853-5541

Coinvestigators:
Dr. Peter Friedland
(415) 497-3728
Dr. Douglas Brutlag
(415) 497-6593
Dr. Laurence H. Kedes
(415) 493-5000 ext. 5318

Services Available

- *Instruments:* Digital Equipment Corporation (DEC)-2060 timesharing computer system, including:
—Central processor, including 2.75-Mwords memory;
—1,000 Mbytes of disk storage;
—LP-20 600 lines/minute line printer;
—TU78 6250/1600 dual density tape drive;
—Telecommunications access via direct dial-in or the Telenet nationwide network.
- *Service Personnel:*
Gene Kromer—Principal Investigator;
Dennis H. Smith—Resource Manager;
Liz Martin—Administrator;
Andrea Gorman—System Administrator;
Terry Friedemann—User Consultant;
Elaine Mansfield—Training Manager;
Tom Bonura—Collaborations.
- *User Lab Space:* Facilities and limited funds available for onsite training and fostering of collaborative research.
- *User Contact Person:* Dennis H. Smith, (415) 328-4870.

Research Emphasis or Application

- *Current Research:* New resource; descriptive material on research capabilities will be provided in subsequent years.
- *Special Features:* The resource staff will provide this support to the BIONET community:
—User consultation by telephone and on-line communication
—Documentation of programs in the core library
—Regional training sessions in use of the software
—Assistance in moving programs into the contributed and core libraries. Using BIONET, investigators will be able to use computer aids in the study of structure/function relationships in nucleic acids and proteins. The computer software to achieve these studies is divided into four categories.
—The Core Library, consisting of nine programs from IntelliGenetics for manipulating and analyzing nucleic acid and protein sequence data, plus additional programs selected by the National Advisory Council (NAC).
—The Data Base Library, containing existing data bases of nucleic acid and protein sequences, including GenBank™, the European Molecular Biology Laboratory (EMBL) database, the National Biomedical Research Foundation (NBRF) library of protein sequences, and VectorBank™.
—The System and Programming Support Library, providing tools for program development, to include programming languages FORTRAN, C, PASCAL, and MAINSAIL, and system utility programs (MLAB, for mathematical modeling, EMACS and TVEDIT for text editing, and SCRIBE for document preparation). Facilities for electronic mail and electronic bulletin boards will also be provided to foster rapid communication and collaboration. KERMIT will be available for file transfer to and from the resource.

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Research Emphasis or Application

—The Contributed Library, for programs contributed and being developed by the BIONET community. Mature versions of such programs will be moved to the Core Library at the discretion of the NAC.

- *New Concept/Capability:* BIONET will provide high-quality, interactive time-sharing access to state-of-the-art computational tools to a potentially very large community of investigators from not-for-profit institutions. By meeting the following goals, BIONET will foster the growth of the field of molecular biology.

The BIONET Resource has three goals:

1. To provide computational assistance in data analysis and problem solving for molecular biologists and researchers in related fields.
2. To serve as a focus for development and sharing of new software tools.
3. To promote collaboration and rapid sharing of information among a national community of scientists.

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Computer Resources

Title of Resource and Principal Investigator

Resource:
Clinical Decision-Making and Artificial Intelligence

Principal Investigator:
Peter Szolovits, Ph.D.
MIT Laboratory for Computer Science
545 Technology Square
Cambridge, Massachusetts 02139
(617) 253-3476

Coinvestigators:
William J. Long, Ph.D.
(617) 253-3508
Stephen G. Pauker, M.D.
(617) 956-5910
Jerome P. Kassirer, M.D.
(617) 956-5930
Benjamin Kuipers, Ph.D.
(617) 253-3510
Alan J. Moskowitz, M.D.
(617) 956-5910

Services Available

- *Software:* Experimental software, developed in conjunction with the three research foci (below) can be made available to collaborators and other interested researchers.
- *Service Personnel:* Proposals for collaboration on the development and application of research ideas are accepted, and project staff are available for collaborative assistance.
- *User Contact Person:* Peter Szolovits, Ph.D., (617) 253-3476.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource-related research project focuses on the development of new methods in three areas: (1) to study and elucidate the knowledge representations and problem-solving methods employed by physicians making difficult management decisions, involving considerable risk and uncertainty, (2) to develop new methods that allow expert programs to explain and justify their conclusions by arguing from fundamental medical facts and principles and reconstructing the path by which those bases have led to the program's recommendations, and (3) to develop and enhance a microcomputer-based decision analysis system for clinical use by physicians, including support for sensitivity analyses and modeling of patient utilities.
- *Current Research:* Analysis of transcripts of expert physicians making patient management decisions; development of transcript analysis methodology; validation of reasoning model developed from transcripts. Development of casual and temporal representations of medical knowledge to support detailed explanations; deriving from interaction with a program's user a model of what the user already knows, to provide better-focused explanations. Produce worked set of decision-analysis examples, including Markov modeling and cost-effectiveness methods; study artificial intelligence methods for helping to formulate decision trees determining patient utility structures.
- *New Concept/Capability:* A better understanding of how expert physicians make difficult management decisions will provide the basis for more effective computer consultation and advising tools. Better methods of computer-generated explanations that are being developed are critical to the utility of many future computer-based decision-making or aiding programs. The project is creating clinically useful tools for implementing patient-specific decision analyses, for use in difficult clinical cases.

[1]Kuipers, B. and Kassirer, J. P., Causal reasoning in medicine: analysis of a protocol. *Cognitive Science* 8:363-385, 1984.
[2]Long, W., Naimi, S., Criscitiello, M., Pauker, S., and Szolovits, P., An aid to physiological reasoning in the management of cardiovascular disease. *Computers in Cardiology*, 1984.
[3]Lau, J., Kassirer, J. P., Pauker, S., Decision maker 3.0: Improved clinical decision analysis by personal computer. *Medical Decision Making* 3:39-43, 1983.

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Title of Resource and Principal Investigator

Resource:
Clinical Decision Systems Research Resource

Principal Investigators:
Harry E. Pople, Ph.D.
University of Pittsburgh
Decision Systems Laboratory
1360 Scaife Hall
Pittsburgh, Pennsylvania 15261
(412) 624-3490

Jack D. Myers, M.D.
University of Pittsburgh
1291 Scaife Hall
Pittsburgh, Pennsylvania 15261
(412) 624-2649

Services Available

- *Software:* CADUCEUS (previously called INTERNIST)—diagnostic program for internal medicine incorporating artificial intelligence techniques.
- *Service Personnel:* Collaboration proposals accepted to interface with other diagnostic systems. A new CADUCEUS system is being implemented locally on a VAX; this system will not be on a national network until the new system is completed.
- *User Space:* No user space provided.
- *User Contact Person:* Harry E. Pople, Ph.D., (412) 624-3490

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* CADUCEUS programs provide access to a comprehensive knowledge base of diseases and symptomatology of internal medicine. Approximately 600 diseases and 4,000 manifestations of disease are organized in a cross-index fashion to provide rapid access to those diseases associated with a given manifestation and those manifestations associated with a given disease. These online data can be accessed for both retrieval and diagnostic consultation.
- *Current Research:* Expanding knowledge base, system modifications, and development of educational and testing subsystems.
- *New Concept/Capability:* A major point of departure for the CADUCEUS diagnostic program is its emphasis on problems of multiple diagnosis. This capability has been achieved by modeling the decision-making processes of experts engaged in the formation of complex clinical hypotheses.

[1]Pople, H. E., Heuristic methods for imposing structure on ill-structured problems: The structuring of medical diagnostics. *Artificial Intelligence in Medicine, AAAS Symposium Series* (Szolovits, P., ed.). Boulder, Colorado, Westview Press, 1982, pp. 119-185.
[2]Miller, R. A., Pople, H. E., and Myers, J. D., INTERNIST-1, an experimental computer-based diagnostic consultant for general internal medicine. *New England Journal of Medicine* 307:468-476, 1982.

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Title of Resource and Principal Investigator

Resource:
GRIP Interactive Graphics System for Molecular Studies

Principal Investigator:
Frederick P. Brooks, Jr., Ph.D.
Department of Computer Science
New West Hall 035A
University of North Carolina
Chapel Hill, North Carolina 27514
(919) 962-2148

Services Available

- *Hardware:* VAX-11/780 with 300 Mb of disk storage, 800/1600 bpi magnetic tape, and 1-Mb memory; Evans & Sutherland PS-300 color vector display; Vector General 3303 vector display; Adage Ikonas high-resolution color frame buffer and graphics processing system; Bausch & Lomb stereoviewer; and a wide variety of interactive input devices (knobs, sliders, joysticks, etc.).
- *Software:* GRINCH, an interactive graphics system for ab initio interpretation of electron density maps (replacing the minimal stage). GRIP-75, an interactive graphics system for display and density-fitting of three-dimensional models of macromolecules and electron-density data, including facilities for measuring and idealizing molecular geometry. Crystallographic programs for isomorphous replacement phasing, electron-density map calculation, structure factor calculation, and structure factor least squares refinement on the VAX-11/780 under the UNIX operating system, all oriented specifically toward macromolecular structures.
- *Service Personnel:* The facility's research assistants help users prepare data for the system, tutor in its use, and accompany production sessions as needed.
- *User Laboratory Space:* Facility is available to users about 150 hours/week. No separate user space is provided.
- *User Contact Person:* Michael Pique, (919) 966-5053.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource enables the biochemist to study three-dimensional computer models of proteins and nucleic acids. Models may be viewed from any angle, at various levels of detail. Conformations may be interactively manipulated via joysticks, etc. Molecular models may be superimposed upon 3-D displays of electron density maps for visual fitting or fit improvement. Maps may be contoured at any level interactively; iterative geometric idealization of models may be done interactively. Photographs, lists of coordinates, and paper plots (skeletal and surface) may be produced.
- *Current Research:* Exploitation of ridge-lines, a new, radically more economical representation for density functions; transfer of molecular software to high-performance work stations, application of advanced graphics technology to molecular problems; display, computation techniques for molecule-molecule docking; unconventional visualizations for molecular structure and properties.
- *New Concept/Capability:* A special feature of the GRIP resource is that the facilities provided are based on observation of the ways many people have tried to use the system. The combination of computer scientists interested in the general problem of computer presentation and manipulation of complex, three-dimensional data structures and biochemists working with real problems has been extremely productive. Some users have been able to complete fits of protein molecules to electron density data without ever building a brass Kendrew model. Fitting-speed improvements 5-13 times those using Richards' box techniques have been reported.

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Title of Resource and Principal Investigator

Resource:
Laboratory of Applied Pharmacokinetics

Principal Investigator:
Roger W. Jelliffe, M.D.
Professor of Medicine
University of Southern California School of Medicine
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(213) 224-7173

Coinvestigators:
Alan Schumitzky, Ph.D.
David Z. D'Argenio, Ph.D.
Darryl Katz, Ph.D.
Mark Milman, Ph.D.
William F. Nicholson, M.D.
(213) 224-7173

Services Available

- *Hardware:* One Decwriter 1200-baud terminal; two Execuport 300-baud terminals; two TI 745 300-baud terminals; one Diablo 300-baud terminal; one Adds variable speed terminal; one automated infusion apparatus; one IBM with 8087 coprocessor; 5MB fixed, 5MB removable head disk; expansion boards; clock and calendar.
- *Software:* ADAPT; USC*PACK; and Automated Drug Infusion Systems. Computer programs for simulation, parameter identification, and "Multiple Model" adaptive control of pharmacokinetic systems. Time-shared computer programs for clinical use in planning, monitoring, and adjusting dosage regimens of digoxin, digitoxin, aminoglycoside antibiotics, lidocaine, procainamide, and theophylline. The programs interface with "smart" infusion apparatus for automated delivery of stepwise tapering infusion protocols to control serum levels of lidocaine and other multicompartment drugs.
- *Special Features:* Systems for optimizing design of pharmacokinetic studies and strategies for therapeutic drug monitoring in patients. Systems for optimal stochastic estimation and control of pharmacokinetic systems for patients and for pharmacokinetic studies.
- *Service Personnel:* Engineers and mathematicians help instruct in use of ADAPT programs for design of pharmacokinetic studies and analysis of pharmacokinetic data, employing least squares and maximum a posteriori probability Bayesian tools; physicians and pharmacists

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Tools for optimal planning, updating, and clinical control of pharmacokinetic systems (drug therapy) in patients. Automated delivery of variable infusion protocols for pharmacokinetic studies and for direct patient care.
- *Current Research:* This laboratory has developed the current ADAPT package of computer programs for design and analysis of pharmacokinetic studies. Optimal sampling strategies can be computed. Data can be analyzed for large, multicompartment models, even in the presence of varying factors such as changing renal function or cardiac output, using either weighted nonlinear least-squares or maximum a posteriori Bayesian methods.

  The USC*PACK programs apply these concepts to optimal planning, monitoring, and adjustment of drug dosage regimens for patient care. They now interface with automatically programmable infusion apparatus for automated delivery of the infusion protocols needed to achieve and maintain stable serum concentration goals for drugs having multicompartment pharmacokinetic behavior. Newer programs for "Multiple Model" adaptive control have now been developed. They now can consider many of the uncertainties in the patient's clinical environment, such as errors in dosage preparation and errors in recording when doses were given, infusions started and stopped, and serum drug levels obtained. Studies are in progress to examine the relative contributions of ward management, dosage formulation, and laboratory assay, and to control the precision of serum drug levels in patients.
- *New Concept/Capability:* Development of new tools for optimal, fully Bayesian analysis and optimal openloop-feedback control of pharmacokinetic and other compartmental systems. Development of approximate optimal closedloop-stochastic control of such systems.

[1]D'Argenio, D. Z., and Schumitzky, A., A program package for simulation and parameter estimation in pharmacokinetic systems. *Computer Programs in Biomedicine* 9:115-134, 1979.
[2]D'Argenio, D. Z., Optimal sampling times for

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Services Available

help teach use of the USC*PACK clinical programs, derived from the ADAPT programs, for optimal planning, monitoring, delivery and adjustment of drug dosage regimens. Informal workshops are given on request.

- *User Laboratory Space:* Space for informal instruction and "hands on" experience is available for research scientists and for interested physicians and pharmacists. Orientation is toward research in adaptive control on the one hand, and toward practical aspects of patient care on the other.
- *User Contact Person:* Roger W. Jelliffe, M. D., (213) 224-7173.

Research Emphasis or Application

pharmacokinetic experiments. *Journal of Pharmacokinetics and Biopharmaceutics* 9:739-756, 1981.

[3]Jelliffe, R. W., Time-shared computer programs for therapeutic drug monitoring and adaptive control of dosage regimens. *Therapeutic Drug Monitoring, Continuing Education, and Quality Control Program,* American Association for Clinical Chemistry, Washington, D.C., June 1981.

[4]Jelliffe, R. W., Pharmacokinetic Dosing Regimens: Implications for Future Technology. *AAMI Technology Assessment Report,* No. 5, pp. 3-6, 1983. Conference on Infusion Technology and Therapy, Arlington, Virginia, 1982.

[5]Jelliffe, R. W., and Nicholson, W. F., Computer assisted therapy and automated infusion apparatus. World Conference on Medical Informatics (Medinfo 83), Amsterdam, Holland, August 22-27, 1983.

[6]Nicholson, W. F., Clinical use of the superposition principle for drugs with linear kinetics. *Clinical Pharmacology and Therapeutics* 33:259, 1983.

[7]Katz, D., and D'Argenio, D. Z., Discrete approximation of multivariate densities with application to Bayesian estimation. *Computational Statistics and Data Analysis* 2:27-36, 1984.

[8]Rodaman, J. H., Jelliffe, R. W., Kolb, E., Tuey, D. B., de Guzman, M. F., Wagers, P. W., and Haywood, L. J., Clinical studies with computer-assisted initial lidocaine therapy. *Archives of Internal Medicine* 144:703-709, 1984.

[9]Schumitzky, A., Milman, M., Katz, D., D'Argenio, D. Z., and Jelliffe, R. W., Stochastic control of pharmacokinetic systems. Submitted for consideration for presentation at meetings of Computer Applications in Medical Care, Baltimore, Maryland, October 23-26, 1983.

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Title of Resource and Principal Investigator

Resource:
Mayo Biomedical Technology Research Computer Facility

Principal Investigator:
Richard A. Robb, Ph.D.
Mayo Foundation
200 First Street, Southwest
Rochester, Minnesota 55905
(507) 284-2511

Services Available

- *Hardware:* Dynamic spatial reconstruction high-speed 3-D imaging system; CDC CYBER 170/720 multiprocessor system; ModComp CLASSIC and Perkin-Elmer 7/32 miniprocessors; DEC PDP 11/23, Zilog 80, and Universe 68 microprocessors; Jupiter-7 graphics processor; Floating Point Systems AP120B and Mercury Zip 32-array processors; and graphic, gray level, and color display systems with 32 access terminals.
- *Software:* FORTRAN, PASCAL, BASIC, COBOL, "C", UNOS (operating system), NOS (Network Operating System), SPSS (Statistical Percentage for the Social Sciences), NSWC/DL (mathematical subroutine library), TIGS (Terminal Interactive Graphics System), and other special purpose statistical and image-processing packages.
- *Service Personnel:* Facility personnel operate system; programming and operation training offered. Collaboration includes service specialties in epidemiology, cardiology, radiology, and cancer.
- *User Laboratory Space:* Limited user space provided.
- *Service Fees:* Charges vary with service provided.
- *User Contact Person:* Richard A. Robb, Ph.D., (507) 284-2511.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource provides dynamic quantitative imaging systems and techniques for analysis of biosystem structures and functions. Investigators studying the relationships between physiological function and anatomic structure can utilize computer-based x-ray and ultrasound scanning systems to record images of organs of the body with high temporal and spatial resolution. A battery of comprehensive analysis and display procedures can be performed on the image data. These same procedures can be applied to light images from a microscope or to closed-circuit television scans of photographic recordings. These methodologies are well suited for accurate, synchronous measurement of the three-dimensional geometry and motion of organs such as the heart and lungs, and for quantitative characterization of tissue properties anywhere in the body.
- *Current Research:* Development, application, and evaluation of x-ray, ultrasound, radionuclide, and NMR quantitative imaging techniques, particularly high-temporal resolution, cylindrical scanning x-ray computerized tomography for noninvasive mathematical vivisection, and dynamic multidimensional display and analysis of bodily organ structures and functions; generation of a comprehensive data base of x-ray, ultrasound, gamma-ray, and NMR projection and reconstruction images of all major organs of the body; three-dimensional dynamic reconstruction of the heart, lungs, and circulation for studies of myocardial and pulmonary stress/strain relationships, regional distribution of myocardial blood flow, detection and quantitation of myocardial infarction, myocardial contractility, circulatory function, regional pulmonary ventilation and perfusion, congenital and acquired heart disease, and automated analysis and quantitation of microscopic structures, including endothelial cells, myocardial tissue constituency, and blood platelet inhibitors and adhesions; correlation of heart, brain, and breast morphology by computerized x-ray and ultrasound tomography, with gross and histological pathology; analysis by ultrasound of tissue characteristics in atherosclerosis and breast cancer; and quantitation of fractional bone volume and mineral content in osteoporosis and in rheumatoid arthritis.

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Research Emphasis or Application

• *New Concept/Capability:* This resource has played a major role in evolution of synchronous dynamic volume x-ray-computed tomography. This capability is based on the concept of true three-dimensional image reconstructions of moving objects from their x-ray projections recorded very rapidly from many angles of view. This concept has been implemented in a new generation x-ray-CT scanner, the Dynamic Spatial Reconstructor (DSR),[1] which produces stop-action (1/100 sec), synchronous volume (240 simultaneous cross sections, each 1-mm thick), high repetition rate (60/sec) reconstructions of any portion of the body. The DSR is particularly suited for dynamic three-dimensional imaging of the heart[2] and measurement of the spatial/temporal distribution of blood flow in any organ region of the body.[3] The system was simulated and designed using the computer resource. The computer resource continues to provide computational support for evaluation and applications of the system, particularly for quantitative analyses and comprehensive, interactive three-dimensional display[4] of the images it produces.

[1]Robb, R. A., Hoffman, E. A., Sinek, L., Harris, L. D., and Ritman, E. L. *Proceedings of the IEEE* 71:308-319, 1983.
[2]Robb, R. A., and Ritman, E. L., *Radiology* 133:655, 1979.
[3]Ritman, E. L., Kinsey, T. H., Robb, R. A., Gilbert, B. K., Harris, L. D., and Wood, E. H., *Science* 210:273, 1980.
[4]Harris, L. D., Robb, R. A., and Ritman, E. L., *Journal of Computer-Assisted Tomography* 3:439, 1979.

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Title of Resource and Principal Investigator

Resource:
Microprocessor Biomedical Technology Resource

Principal Investigator:
Robert L. Schoenfeld, D.E.E.
Rockefeller University
1230 York Avenue
New York, New York 10021
(212) 570-8613

Services Available

• *Hardware:* IBM-PC and XT computers, PC-MULTIBUS interbus communication and Bit3 expansion chassis; Intel single board computers with 8086, 80186, and 80286 processors; 3COM Etherlink/IBM PC interface; Intel 286/380 computer development system; Intel Series III development system; IBM PC compatible peripheral modules including IEEE 488 interface, Data Translation DT2801A data acquisition board, Okidata and Epson printers, EPROM programmer, analog-digital conversion, digital input-output, and programmed clock modules. Prototyping support includes: Texas Instruments 7000 emulator, Microtek 80186/188 in circuit emulator, Intel Universal Programmer. Supplements: Amdek, Sony, and Panasonic monitors, and Zenith Z-19 terminals and PET Commodore computer systems.

• *Software:* Operating system support for the various development stations with Xenix 286, Venix, and AMPOS. Additional operating system resources include: RMX86, CPM80, CPM86, and ISIS. High-level programming language support for C, PL/M86, PL/M80, PL/M51, MODULA 2, PASCAL, FORTRAN; assemblers for a variety of microprocessors.

• *Service Personnel:* The resource provides consultation, design, development, and programming in the application of computer technology to the laboratory projects of biomedical collaborators. A project engineer is assigned to each accepted collaborative endeavor. Users are expected to pay hardware and construction

Continued on next page

Research Emphasis or Application

• *Data Achievable with Resource's Methodologies:* This resource provides a computer engineering capability for biomedical investigators who require techniques for patterned stimulation of sensory modalities or activity with concomitant sensing of neurophysiologic and pharmacologic/biochemical data or physical activity. Microprocessor-based electronic technology is combined with programming methodology and software tools to provide laboratory microcomputer systems for experimental control and data acquisition. Visual and electrophysiologic stimulators, histogram and neural signal averagers, and general purpose laboratory computer systems may be replicated.

• *Current Research:* Experiments involving computer-based measurements of activity: food and drink ingestion; heart rate; display, processing, and analysis of EKG waveforms; nerve and muscle activity; spectral analysis and stimulus patterning in nerve and muscle recording; neurophysiologic and psychological experiments; control and data acquisition in analytical chemistry and mass spectrometry. Visual, postural, and neuromuscular research. Exploration of clinical applications including rehabilitative medicine, prosthesis, and scoliosis.

• *New Concept/Capability:* The inherent parallelism of component tasks in biomedical laboratory control, data acquisition, and display can be exploited with multiprocessor-based computer hardware and an appropriate operating system, AMPOS. The current implementation of this system includes an IBM XT personal computer; a PC to Multibus adapter; and a cable to a separate chassis containing Intel single board computers, each performing a separate I/O, display, or computational task. The control and synchronization of these tasks is effected through a UNIX* process in the IBM PC host. The bandwidth of the essentially concurrent activities on the multiple subsidiary processors is not reduced significantly by the supervisory actions of the host. Capabilities also exist for developing a variety of low-cost, light weight, portable embedded processors for pharmacologic, physiologic, and chemical instrumentation applications, as well as for medical prosthesis.

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Services Available

costs where applicable. A laboratory course will be offered each spring and in accelerated form during the summers of 1986 and 1987.

- *User Laboratory Space:* The physical facilities support the design, development, and programming of laboratory computers ranging in complexity and generality from multiprocessor based systems supporting the experimental activity of a group, to "chip" systems embedded in a single instrument.
- *User Contact Person:* Gordon Silverman, Ph.D., (212) 570-8612.

Research Emphasis or Application

[1]Schoenfeld, R. L., A programming discipline for laboratory computing. *IEEE Transactions on Biomedical Engineering*, 5 May, 1983.
[2]Silverman, G., Automation in the biomedical laboratory. *IEEE Transactions on Biomedical Engineering*, December 1984.
[3]Harrison, M., and Smith, O., The AMPOS Multiprocessor System. *IEEE Micro*, 1984 (in press).

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Title of Resource and Principal Investigator

Resource:
PROPHET National Time-Sharing Computer Resource

Program Director:
Charlotte Hollister
Bolt Beranek and Newman, Inc.
50 Moulton Street
Cambridge, Massachusetts 02238
(617) 491-1850

Services Available

- *Hardware:* DEC KL-10 with TOP-10 time-sharing monitor, 1 million words of memory, online and archival disk storage, magnetic and DEC tape drives, line printer, and plotter.
- *Software:* PROPHET System (chemical/biological handling system). Languages include PL/PROPHET (user programming language), FORTRAN, SAIL, BASIC, and MACRO. Packages include PROPHET Public Procedures (user applications), MLAB (math-modeling/curve fitting), CLAB (cluster analysis/pattern recognition), CELLSIM (cell kinetics), ORTEP (molecular graphics), HUMAN (simulation package), BMDP (statistics), Cambridge crystal file (x-ray structures), Brookhaven protein file (x-ray structures), and QCFF and MM2 (molecular modeling).
- *Access:* National access provided by DRR via WATS telephone numbers, Telenet, and direct lines.
- *Service Personnel:* Systems analyst/programmers for system maintenance/development; applications analyst/programmers for new feature development; user representatives for periodic visits to user sites and phone consultations; and computer operators for 24-hour/day computer service.
- *User Laboratory Space:* NIH Division of Research Resources (DRR) supplies all equipment for each user site, which provides its own lab space. Occasionally, space is available at current user sites for visiting scientists.

Continued on next page

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* PROPHET is a time-sharing resource developed specifically for use by pharmacologists, medicinal chemists, and other biomedical scientists studying the interactions of chemical and biological systems. PROPHET features tools for data management, data analysis, and molecular modeling. Communicating with PROPHET via higher level, English-like commands, scientists can create, modify, display, and manipulate a variety of standard entities, such as tables, graphs, and molecular structures.
- *Current Research:* Currently, more than 800 researchers at 40 academic, governmental, and commercial research institutions throughout the United States are using PROPHET in studying interactions of chemical and biological systems.
- *New Concept/Capability:* Tools to assist in sequencing analysis of proteins and DNA; tools for pharmacokinetic modeling and survival analysis; ability to manipulate more than one molecular structure in the same coordinate space and to visualize the spatial relationship; a new user-friendly interface to the Cambridge Crystal File Data Base; color graphics and plotting; ability to access using many types of display terminals and to communicate with micro- and minicomputers located at the user's institution. PROPHET is the host for the NIH-sponsored GenBank™, a genetic sequence data bank.

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Services Available

- *Service Fee:* Schedule available; actual cost but substantial subsidy for nonprofit users.
- *User Contact Persons:*

—*Hardware:* Leonard M. Hantman, Bolt Beranek and Newman, Inc., 50 Moulton Street, Cambridge, Massachusetts 02238, (617) 491-1850.

—*Software:* Howard Bilofsky, Ph.D., Bolt Beranek and Newman, Inc., 50 Moulton Street, Cambridge, Massachusetts 02238, (617) 491-1850. PROPHET staff also available via PROPHET Help Line, (617) 491-7128.

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Computer Resources

Title of Resource and Principal Investigator

Resource:
Protein Identification Resource

Principal Investigators:
Robert S. Ledley, D.D.S.
National Biomedical Research Foundation
Georgetown University Medical Center
3900 Reservoir Road, N.W.
Washington, D.C. 20007
(202) 625-2121

Winona C. Barker, Ph.D.
National Biomedical Research Foundation
Georgetown University Medical Center
3900 Reservoir Road, N.W.
Washington, D.C. 20007
(202) 625-2121

Coinvestigators:
Lois T. Hunt, Ph.D.
David G. George, Ph.D.
(202) 625-2121

Services Available

- *Hardware:* DEC VAX 11/780 with 8 megabytes of high-speed memory, 734 megabytes of disk storage, 800/1600-bpi magnetic tape unit, card reader, and 22 hard-wired terminals including two CIT-414A graphics terminals; Printronix 300 lineprinter; TEXAC special-purpose image-analyzer computer, which performs extremely rapid whole-picture operations; DEC stand-alone word processor; and various microcomputers.
- *Software:* FSQ (Protein Sequence Query) and NAQ (Nucleic Acid Query), main retrieval programs allowing retrieval based on text, sequence, or sequence properties, as well as a variety of sequence manipulation and display routines; SCAN, rapid data base searching procedure for identically matching segments; SEARCH, data base searching program allowing mismatches; DFASTP, data base searching program allowing mismatches and insertion/deletion events;[1] ALIGN, pairwise sequence alignment program providing statistical evaluation of sequence similarity; RELATE (DOTMATRIX), segment comparison programs for locating local regions of sequence similarity; programs for producing sequence alignments and phylogenetic trees; and a variety of other sequence examination and manipulation programs. Programs are written in VAX FORTRAN-11 and are available for VAX/VMS systems.

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* This resource provides the facilities necessary for the identification of unknown proteins based on protein sequence, partial sequence, or amino acid compositional information. Users can determine whether the protein sequence has previously been reported, can identify closely related or homologous protein sequences, can identify sequence regions that are similar to conserved active or functional site patterns, or can browse through the Protein Sequence Information System and obtain more general information. The resource also has the capability to correlate protein and nucleic acid sequence information and thus provide the facility for the identification of proteins based on nucleic acid sequence information.
- The primary research of this resource is the development, examination, and application of methods for protein sequence identification. In addition to the classic methods for protein identification based on global sequence similarity,[2] methods of identification based on less well determined criteria are being examined. Examples of such criteria are: the existence of localized domains of sequence similarity, structural similarity, functional similarity, prosthetic group binding, and other ancillary protein information.
- *New Concept/Capability:* This resource maintains the largest and most comprehensive protein sequence data base in the world.[3,4,5,6] The computer system is specifically designed to use the data organizational structures efficiently (sequence homology, physical and functional properties of the proteins, taxonomic classification, etc.) for information retrieval and access and thus provides an extremely powerful Protein Sequence Information System.[7] In conjunction with the sequence and amino acid composition searching routines, this system provides the capability for the examination and identification of new and unknown protein sequences and can also provide insight into problems concerning protein structure and function.

[1]Wilbur, W. J., and Lipman, D. J., *Proceedings of the National Academy of Sciences* 80:726-730, 1983.
[2]Dayhoff, M. O., Barker, W. C., and Hunt, L. T., *Methods in Enzymology* 91:524-545, 1983.

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Services Available

- *General Features:* Electronic mail, text editing, interactive user help, on-line documentation.
- *Special Features:* Protein Sequence Data Base, National Biomedical Research Foundation; Nucleic Acid Sequence Data Base, National Biomedical Research Foundation; GenBank® Nucleic Acid Sequence Data Base, Bolt Beranek and Newman, Inc.; EMBL Nucleotide Sequence Library, European Molecular Biology Laboratory; and portions of the Brookhaven Protein Data Bank (Crystallography), Brookhaven National Laboratory. Programs and data are available on 1600 or 800 bpi tapes. Online computer access: National access via TYMNET. Local access via public phone lines; three 1200 baud and three 300 baud lines.
- *Service Personnel:* Resource staff are available to perform special computer services and to collaborate on projects that may augment the facilities of the resource.
- *User Laboratory Space:* Visiting Scientist Program: Limited funding and in-house space are available for visiting research scientists on a short-term basis. Service Fees: Online access is free to researchers engaged in non-commercial work at tax-exempt institutions. Fees to others are based on connect time, CPU time, and network access charges. Fees for special computer services vary.
- *User Contact Person:* Kathryn E. Sidman, B.S., general information and services; Winona C. Barker, Ph.D., and Lois T. Hunt, Ph.D., research or scientific information; David G. George, Ph.D., computer system information; (202) 625-2121.

Research Emphasis or Application

[3]Dayhoff, M. O., ed., *Atlas of Protein Sequence and Structure,* vol. 5, National Biomedical Research Foundation, Washington, D.C., 1972.
[4]Dayhoff, M. O., ed., *Atlas of Protein Sequence and Structure,* Vol. 5, suppl. 1, National Biomedical Research Foundation, Washington, D.C., 1973.
[5]Dayhoff, M. O., ed., *Atlas of Protein Sequence and Structure,* Vol. 5, suppl. 2, National Biomedical Research Foundation, Washington, D.C., 1976.
[6]Dayhoff, M. O., ed., *Atlas of Protein Sequence and Structure,* Vol. 5, suppl. 3, National Biomedical Research Foundation, Washington, D.C., 1979.
[7]Orcutt, B. C., George, D. G., and Dayhoff, M. O., *Annual Review of Biophysics and Bioengineering* 12:419-441, 1983.

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Title of Resource and Principal Investigator

Resource:
Research in VLSI Systems for Biomedical Research

Principal Investigator:
Charles E. Molnar, Sc.D.
Computer Systems Laboratory
Washington University
724 South Euclid Avenue
Lopata Hall, Room 509
Campus Box 1099
St. Louis, Missouri 63110
(314) 454-3969

Services Available

- As a resource-related research project, our primary goal is to add to the capabilities of the Washington University Resource for Biomedical Computing, which supports a large variety of biomedical application projects of both local and national scope. Only services specific to this project will be listed here.
- *Hardware:* A VAX-11/750, running UNIX software licensed by us through the University of California at Berkeley, is the primary computing tool supporting both very large-scale integrated (VLSI) design and exploratory application research. This system is equipped with AED-512 color-graphics displays and multicolor plotters, primarily intended as design work stations. We also operate several MMS-X high-performance calligraphic display systems, and plan to modify them for use as display devices for specialized computer systems. We also provide hardware and software support for microprocessor systems, based on the Motorola MC68000, intended to be used as component processors embedded in specialized multiprocessor systems.
- *Software:* Most current work uses software development tools that run on the VAX under UNIX, with cross-compilation of C programs for the MC68000.
- *Service Personnel:* Our research and development activities have not yet matured to the point that we can offer organized services to the biomedical community. Initial steps toward reaching a broader community include our giv-

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Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The research aim is to provide available tools and methods for the design of VLSI circuits for application to biomedical research problems. This work will provide unique and improved capabilities for data acquisition, processing, display, modeling, and control applications in biomedical science. Current application projects and collaborations include: (1) development of new circuits to improve in a practical way the accuracy of radiation treatment planning calculations; (2) study of improved methods for collecting and analyzing time-of-flight positron emission tomography (PET) data; (3) development of concepts and equipment to support the design of improved digital hearing aids; (4) study of highly parallel algorithms and system architectures for molecular modeling and drug design; (5) design of specialized circuits for digital communication and information processing; (6) modeling of mechanical and neural processes in the cochlea.
- *Current Research:* Development of computer-aided design (CAD) tools for specifying and designing complex circuits and systems that can be fabricated using modern silicon technology. The tools include computer programs and specialized peripheral equipment developed in projects at other universities sponsored primarily by the National Science Foundation and the Department of Defense, as well as new tools derived from our long-term research in macromodular computer systems. We are developing methods for specifying the interactions between modules making up a computing system in terms of discrete event-based models that suppress the details of space and time, but which will serve as a starting point for a rigorous design methodology leading to circuit designs that can be proved to be reliable. Our methods do not require the availability of a system-wide clock. They allow very straightforward specification of structures with computational concurrency. Examples of structures that we can design with our methods include speed-independent bus structures capable of one-to-many transmission,[1,2] asynchronous crosspoint switch structures,[3] and highly concurrent asynchronous pipeline structures.

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Services Available

ing short courses in VLSI design, oriented toward potential users from the biomedical research community, and making presentations to specialized audiences calling their attention to the potential of VLSI.[4]

- *User Laboratory Space:* Space and informal instruction are available for visiting scientists and research colleagues who wish to learn to use our design aid facilities.
- *User Contact Person:* Frederick U. Rosenberger, D.Sc., (314) 454-3395.

Research Emphasis or Application

Related research and development provide supporting tools for the general-purpose computational tasks usually closely associated with highly specialized computing and data collecting tasks, and techniques and equipment for high-performance display of results and ongoing computations.

- *New Concept/Capability:* The most novel aspect of our work is the development of a comprehensive design methodology for asynchronous systems, which are generally regarded as too difficult to design and too inefficient to implement. We feel that much of the difficulty that has been experienced in using asynchronous techniques has resulted from misapplication of models and tools developed originally for the description of rigidly clocked structures. The simplicity with which the intercommunication of asynchronous modules can be specified in a rigorous way makes them especially valuable for the construction of specialized systems for which design costs usually dominate the costs of system construction.

[1]Sutherland, I. E., Molnar, C. E., Sproull, R. F., and Mudge, J. C., The TRIMOSBUS. *Proceedings of the Conference on Very Large Scale Integration, Architecture, Design Fabrication,* California Institute of Technology, Pasadena, California, January 22-24, 1979, pp. 395-427.
[2]Bosch, W. R., A TRIMOSBUS-based multimicroprocessor system prototype. Master of Science thesis in computer science, Sever Institute, Washington University, St. Louis, Missouri, 1983.
[3]Wann, D. F., and Franklin, M. A., Asynchronous and clocked control structures for VLSI based interconnection networks, *IEEE Transactions on Computers.* In press.
[4]Molnar, C. E., VLSI systems for time-of-flight PET. *Proceedings of the International Workshop on Time-of-Flight Tomography,* IEEE Catalog No. 82CH1791-3, Washington University, St. Louis, Missouri, pp. 167-170, 1982.

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Title of Resource and Principal Investigator

Resource:
Resource for Biomedical Computing

Principal Investigator:
Lewis J. Thomas, Jr., M.D.
Washington University School of Medicine
700 South Euclid
St. Louis, Missouri 63110
(314) 362-2135

Services Available

- *Hardware:* Two dual-processor systems for high-speed ECG processing; an image-processing development system (DEC PDP-11/34 with Lexidata 3400-raster-scan processing and display unit); one PDP-11/34 used in engineering design for hardware development, software system development, and application support; one Masscomp MCS-500 graphics work station; microprocessor development support; an MMS-X system supporting high-performance graphics; a DEC LSI-11/23 processor interfaced to an FPS model 100E array processor and associated ultrasonic systems for tomographic scanning (transmission and backscatter); a 30-port TERRANET local-area network for distributed access to heterogenous computing resources; and a dual-cable broadband network for the acquisition and distribution of radiologic image data.
- *Software:* RSX-11, RT-11, and UNIX operating systems supporting BASIC, FORTRAN, MUMPS, C, PASCAL, and FOCRAS (FORTRAN-based cross assembler for microprocessor support).
- *Service Personnel:* Resource personnel perform all operations. Consultation and collaboration are offered for participants in the resource program.
- *User Laboratory Space:* Space is made available for personnel engaged in collaborative research, but many activities are located in the collaborators' laboratories or clinical facilities.
- *User Contact Person:*
L. J. Thomas, Jr., M.D., (314) 362-2135.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The laboratory develops models, algorithms, and specialized computer systems for use in basic and clinical research. Examples of data achievable with systems designed by the resource are: high-speed arrhythmia analysis of ambulatory ECG recordings; tissue characterization from ultrasonic measurements of attenuation and backscatter; counts of individual silver grains in autoradiographs; regional tracer estimates enhanced by time-of-flight measurements in positron emission tomography of heart and brain; and automated reading of electrophoretic gels for DNA mapping.
- *Current Research:* Emphasis is on quantitative biomedical imaging for a broad range of research projects, including modification of infarct size; ultrasonic tissue characterization; emission tomography of heart and brain; algorithms for automated analysis of electron-microscopic autoradiographs; algorithms and custom IC designs for coping with inhomogeneities and irregular fields in radiation treatment planning; computer-based chromatic perimetry; physical mapping of DNA; microprocessor development support; local-area networks; unified approaches to data acquisition, processing, and display; physiological signal processing; and algorithms for quantitative information extraction from biomedical images.
- *New Concept/Capability:* We use digital computing systems and devices to solve biomedical research problems requiring close coupling between the computer and the experiment. Emphasis is on collaborative research with biomedical investigators rather than on fee-for-service arrangements. No particular biomedical research area is given preference in the collaborations, which span basic science through clinical studies. New concepts range from novel approaches to digital communication, through explorations of demanding algorithms for analyzing biomedical images and signals, to the development of specialized devices for solving problems intractable by conventional methods.

[1]Gran, A. J., Beecher, D. E., and Olson, M. V., Computer-based image analysis of one-dimensional electrophoretic gels used for the separation of DNA restriction fragments. *Nucleic Acids Research* 12:473-491, 1984.

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Research Emphasis or Application

[2]Larson, K. B., Biological Transport of Radiotracers. *Journal of Nuclear Medicine* 25:543-544, 1984.
[3]Miller, J. G., Ultrasonic characterization of myocardial tissue. *Frontiers of Cardiology for the 80's*, Academic Press, London, 311-312, 1983.
[4]Shum, C. D., Cox, J. R., and Blaine, G. J., Design analysis of a wide-band picture communication system. *IEEE International Symposium on Medical Images and Icons, ISMII '84*, 66-77, 1984.
[5]Snyder, D. L., Parameter estimation for dynamic studies in emission-tomography systems having "list mode" data. *IEEE Transactions on Nuclear Science* NS-31:2, 925-931, April 1984.
[6]Thomas, L. J., and Miller, J. P., Long-term ambulatory ECG recording in the determination of antidysrhythmic drug efficacy. NIH Workshop on Pharmacology of Antidysrhythmic Drug Therapy, Bethesda, Maryland, September 1982. *Perspectives in Cardiovascular Research*, Volume 10: Clinical Pharmacology of Antiarrhythmic Therapy, B. R. Lucchesi, J. V. Dingell, and R. P. Schwartz, Jr., eds., Raven Press, 249-265, 1984.
[7]Wong, J., Slessinger, E. D., Stein, D., Rosenberger, F. U., and Purdy, J. A., Implementation and verification of a new CT-based 3-dimensional photon dose calculation algorithm. *Medical Physics* 10:543, 1983.

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Title of Resource and Principal Investigator

Resource:
Rutgers Research Resource on Artificial Intelligence in Medicine

Principal Investigators:
Casmir A. Kulikowski, Ph.D.
Solomon M. Weiss, Ph.D.
Department of Computer Science
Hill Center, Busch Campus
Rutgers University
New Brunswick, New Jersey 08903
(201) 932-3546/2768

Services Available

- *Hardware:* At Rutgers: DEC 2060T processor with 1.5 M words of MOS memory, 120 M words of RPO6 file storage, and two industry-compatible tapes, running the TOPS 20 operating system; 96 terminals to access these systems plus hard copy, printer, and graphics. SUMEX-AIM facility also available to investigators.
- *Software:* Languages include BASIC, BLISS 10, FORTRAN, FUZZY (a language for reasoning about imprecise concepts), LISP (a number of dialects), MACRO-10, MAINSAIL, PASCAL, SAIL, SETL, and SITBOL. Access is local, via phone and direct lines and remote, via the TYMNET network and the ARPANET.
- *Service Personnel:* Staff members at the resource organize an annual workshop on artificial intelligence in medicine (AIM). Consultation and collaboration are available, especially through common access to the resource computer for projects reviewed and approved by the AIM-executive committee.
- *User Laboratory Space:* Terminals and support hardware.
- *User Contact Person:* Charles Hedrick, Ph.D., (201) 932-3088.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Development of artificial intelligence methods for biomedical research and clinical decision-making; computer sharing in system development and research.
- *Current Research:* Medical modeling and expert decision-making systems (consultation systems: EXPERT/rheumatology, microprocessor EXPERT/serum protein, EXPERT systems in ophthalmology); general knowledge representation schemas; learning systems, such as SEEK, and time-oriented clinical data base analysis/interpretation programs; artificial intelligence research on representations, reasoning, and system development (inductive inference and theory formation, development of AI languages).
- *New Concept/Capability:* EXPERT is a method for incrementally building biomedical consultation reasoning models using highly efficient reasoning strategies based on the expertise of specialists. EXPERT has been used to develop a large-scale consultation system in rheumatology, in collaboration with the University of Missouri in Columbia, and consultation models in ophthalmology and clinical pathology. With an automatic translation process, we developed the first expert system on a chip. The system was incorporated within a scanning densitometer for analysis of serum protein electrophoresis. The general EXPERT schema recently has been implemented on a microprocessor-based system (MC68000), making it feasible to increase the dissemination of AI methods in the biomedical community. Knowledge acquisition, model-driven, and empirical learning procedures, as well as strategies for planning treatment are being investigated to extend the EXPERT methodology.

[1]Kulikowski, C., Artificial intelligence methods for medical consultation. *IEEE Transactions on Pattern Analysis and Machine Transaction:* PAMI-2, 1980.
[2]Kastner, J. K., Weiss, S., and Kulikowski, C., Treatment selection and explanation in expert medical consultation: Application to a model of ocular herpes simplex. *MEDCOMP '82,* 420-426, 1982.
[3]Politakis, P., and Weiss, S., Using empirical analysis to refine expert system knowledge bases. *Artificial Intelligence Journal* 22:23-48, 1984.
[4]Weiss, S., Kulikowski, C., and Galen, R. S., Developing microprocessor-based expert models for instrument interpretation. *Proceedings of the Seventh International Joint Conference on AI,* Vancouver, pp. 853-855, 1981.

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Computer Resources

Title of Resource and Principal Investigator

Resource:
Simulation Preresource for Stochastic Population Models

Principal Investigator:
Eugene Ackerman, Ph.D.
Health Computer Sciences
University of Minnesota
Box 511 Mayo Memorial Building
420 Delaware St. S.E.
Minneapolis, Minnesota 55455
(612) 373-5616

Coinvestigators:
Elving Anderson
Jose Barbosa
Alan Bender
Lila Elveback
John Fox
David Greenberg
Lael Gatewood
Tom Kottke
Ira Longini
Denton Peterson
Stephen Rich
Susan Seaholm

Services Available

- *Instruments:* DEC VAX 11/750 with VMS Operating System; DEC PDP 11/34A with RSX11-M; Assorted terminals and printers; DECNET; Dialup access.
- *Special Features:* VESPERS programming system for simulation of disease in structured human populations, including interactive facilities for population and parameter specification, multiple-run generation, and sensitivity analyses. Models and parameter sets for simulation of epidemics due to viral infections, including vaccination and behavioral modification programs. Models of primary and secondary interventions against chronic disease to illustrate possible control mechanisms. IMSL and genetic analysis packages (PAP, LIPED, POINTER) for further data analysis.
- *Service Personnel:* Limited support personnel available for assistance with modeling, data analysis, and resource expansion.
- *User Laboratory Space:* Terminals, dialup ports, work space, and documentation available.
- *User Contact Person:* Susan Seaholm, M.S., (612) 373-0335; Laei C. Gatewood, Ph.D., (612) 373-0325.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Infectious Disease Simulations: Age and population group-specific attack rates and time-course of epidemic spread in simulated populations under varying background conditions and prevention measures. Chronic Disease Simulations: Incidence and prevalence rates of chronic disease morbidity, mortality, and case fatality can be simulated and compared to actual population figures. Genetic Simulations: Distributions of expected pedigree structure under proposed genetic models of disease.
- *Current Research:* VESPERS System: Development of VESPERS for VAX computers; superior simulation system programming and preresource management tools, online help, and documentation; parameter sensitivity algorithms and program systems. Infectious Disease Simulations: Viral interference models involving reduced immunity and reduced shedding; vaccination studies; nursing home population simulations. Chronic Disease Simulations: Conversion of VESPERS simulation system for modeling coronary heart disease and comparing models of changing disease rates over time in known populations such as North Karelia, MRFIT, and Minnesota Heart Survey. Genetic Simulations: Development of genetic simulation models for quality control of genetic analysis programs, both for segregation and linkage analysis; development of tools to input model specifications from limited data analysis, and compare sibling and progeny distributions obtained from simulations in which these are known.
- *New Concept/Capability:* VESPERS is an interactive, user-friendly system for discrete micropopulation Monte Carlo simulations of disease in structured populations. The richness, detail, and flexibility of the computer programs and underlying models allow a variety of disease types, populations, and prevention programs to be studied, with extensive reporting available. Viral infections are simulated via contact between infected and susceptible individuals in a variety of pseudosociological mixing groups; states of infectivity include latency, incubation, infectivity with or without symptoms, and withdrawal from some mixing modes; mid-epidemic parameter changes are allowed in order to simulate behavioral changes and vaccination. Chronic disease can be simulated either as snapshots, corresponding to population surveys, or with cohorts as in longitudinal studies. Genetic data simulated may be interfaced with genetic analysis packages such as PAP, LIPED, and POINTER.

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Title of Resource and Principal Investigator

Resource:
Simulation Resource in Mass Transport and Exchange

Principal Investigator:
James B. Bassingthwaighte, M.D., Ph.D.
Center for Bioengineering WD-12
University of Washington
Seattle, Washington 98195
(206) 545-2005

Coinvestigators:
E. O. Feigl
K. A. Krohn
M. M. Graham
T. K. Lewellen
P. J. Kelly
C. Y. Wang
A. Goldstein
C. Goresky
H. Sparks
E. N. Lightfoot
B. Winkler
J. Kulikka

Services Available

- *Hardware:* Data General MV/1000 computer system with 4 Mbytes of memory, 708 Mbytes of disk storage, 1600/800 bpi magnetic tape drive, 1200/300 baud telephone ports, graphics terminals, Versatec printer/plotter, and character printer.
- *Software:* AOS/VS and MV/UX (a Data General hosted-UNIX) operating systems supporting FORTRAN, RATFOR, and C compilers.
- *Special Features:* Simulation Interface, SIMCON.
- *Service Personnel:* Facility personnel available to assist in modeling and fitting models to data.
- *User Laboratory Space:* Terminals available.
- *User Contact Persons:* James B. Bassingthwaighte, M.D., Ph.D., (206) 545-2005/2012; Dean Pentcheff, (206) 545-7433; Richard King, (206) 545-2007/7433.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Estimates of conductances of capillary and cell membranes, volumes of distribution, and regional flows in organs. Identification of receptor affinities and metabolic rates in intact organs via various tracer techniques.
- *Current Research:* Development of an interactive simulation system that permits formulation of models without programming. This is accomplished through the use of menus, automated optimization of model parameters to fit models to data, automated model selection and reduction during analysis, and integration of advanced graphic techniques for the display of results of simulation analysis. Application areas include transport of hydrophilic molecules in microvasculature, simulation analysis for estimation of tracer uptake and deposition in cells as indicators of regional blood flow and cellular function, and analysis of lipoprotein kinetics.
- *New Concept/Capability:* Development of logical structures for experiment design and analysis. This includes approaches automating the fitting of models to data, assessing the goodness-of-fit, choosing the numerical approximations, and in some situations choosing the form of the model.

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Computer Resources

Title of Resource and Principal Investigator

Resource:
Special Research Resource for Biomolecular Graphics

Principal Investigator:
Robert Langridge, Ph.D.
Computer Graphics Laboratory
Department of Pharmaceutical Chemistry
School of Pharmacy
University of California, San Francisco
San Francisco, California 94143
(415) 666-2630

Services Available

- *Hardware and Software:* Two Digital Equipment Corporation VAX-11/750 computers, running under Bell Laboratories UNIX time-sharing operating system. Calligraphic displays available on Evans and Sutherland Color Picture System 2 (color and black-and-white), and Picture System 300 (black-and-white). Main software package is MIDAS (Molecular Interactive Display and Simulation System).
- *Special Features:* Several molecules up to over 100,000 molecular weight can be represented as line drawings and/or surfaces and manipulated selectively and interactively in real-time as the user chooses, to adjust spatial orientation, to display all or a portion of the molecule(s), to display selected regions, to change labeling, to select rotation sites, to calculate distances, and to illustrate physical properties such as charge distributions.[1,2,3]
- *Service Personnel:* Limited staff available to teach, demonstrate, and collaborate, and to supply special purpose software.
- *User Laboratory Space:* A very limited amount is available for use of graphics.
- *User Contact Person:* R. Langridge, Ph.D., (415) 666-2630; T. E. Ferrin, (415) 666-1100.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* The emphasis of this resource is on the structures and interactions of large biological molecules, both with each other and with smaller molecules such as drugs and substrates. Since the three-dimensional color display and the coordinates are directly related, the manipulation of the model is reflected directly in the picture viewed on the screen.[1]
- *Current Research:* Current cooperative and collaborative research includes studies of drug and protein interactions with DNA; thyroxine analog interactions with the thyroid hormone carrier, prealbumin;[4] and dihydrofolate reductase inhibitors.[5] Considerable emphasis is also placed on the user interface since a crucial element in this work is not only the hardware or the software but also the enlightened user.
- *New Concept/Capability:* The introduction of real-time, interactive, three-dimensional color at this laboratory[1] had a considerable impact, not only on work at the laboratory, but also in stimulating others in industry and at universities to propose and install similar hardware. The laboratory's software developments that complement this powerful hardware include selective coloring of large molecules, nonreal-time solvent-accessible surfaces, real-time Van der Waals surfaces[3] (color coded as desired), and real-time stereoviewing.

[1]Langridge, R., Ferrin, T. E., Kuntz, I. D., and Connolly, M. L., Real-time color graphics in studies of molecular interactions. *Science* 211:661-666, 1981.
[2]Weiner, P. K., Langridge, R., Blaney, J. M., Schaefer, R., and Kollman, P. A., Electrostatic potential molecular surfaces. *Proceedings of the National Academy of Sciences* 79:3754-3759, 1982.
[3]Bash, P. A., Pattabiraman, N., Huang, C., Ferrin, T. E., and Langridge, R., Van der Waals surfaces in molecular modeling: An implementation using real-time computer graphics. *Science* 222:1325-1327, 1984.
[4]Blaney, J. M., Jorgensen, E. C., Connolly, M. L., Ferrin, T. E., Langridge, R., Oatley, S. J., Burridge, J. M., and Blake, C. C. F., Computer graphics in drug design: Molecular modeling of thyroid hormone-prealbumin interactions. *Journal of Medicinal Chemistry* 25:785-790, 1982.
[5]Hansch, C., Li, R., Blaney, J. M., and Langridge, R., Comparison of the inhibition of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase by 2, 4-diamino-5-(substituted-benzyl) pyrimidines: Quantitative structure-activity relationships, x-ray crystallography, and computer graphics in structure-activity analysis. *Journal of Medicinal Chemistry* 25:777-784, 1982.

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Title of Resource and Principal Investigator

Resource:
Stanford University Medical Experimental Computer Resource—Artificial Intelligence in Medicine (SUMEX-AIM)

Principal Investigator:
Edward A. Feigenbaum, Ph.D.
Heuristic Programming Project
Department of Computer Science
701 Welch Road, Building C
Stanford University
Palo Alto, California 94304
(415) 497-4879/4878

Coinvestigator:
Edward H. Shortliffe, M.D., Ph.D.
Division of General Internal Medicine-TC135
Stanford University Medical Center
Stanford, California 94305
(415) 497-6979

Services Available

- *Hardware:*

—Digital Equipment Corporation (DEC) 2060 central processor with TOPS-20 time-sharing monitor, 2 megawords (36 bit) of high-speed memory; 1.2 gigabytes of disk storage; two 9-track 1600/6250-bpi industry-compatible tape units; and a line printer.

—DEC 2020 computer with TOPS-20 time-sharing monitor, 512-K words of high-speed memory; 176 megabytes of disk storage; one 9-track 800/1600 bpi industry-compatible tape unit for AI program testing and demonstration.

—Powerful LISP-based work stations with high-resolution bitmap displays for research and development of AI expert systems. Rutgers-AIM facility also available to investigators.

- *Software:* Languages include FORTRAN, BASIC, MACRO, SAIL (15), INTERLISP, UCILISP, MACLISP, PSL, C, PASCAL, BCPL, PDP-11 cross assembler, MAINSAIL, and SITBOL. Packages include OMNIGRAPH (graphics support), MLAB (mathematical modeling), SPSS and STP (statistical packages), FLECS (flexible FORTRAN precompiler), and SCRIBE (document preparation system).
- *General Facilities:* Electronic mail and inter-user communication; network and telephone file transfer; text-editing, searching, and compilation; and online documentation and interactive user help. Annual workshop on artificial intelligence in medicine (AIM) in collaboration with Rutgers-AIM Resource.

Continued on next page

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* SUMEX-AIM is a national resource for applying symbolic computing in knowledge-based programs that emulate expert problem-solving capabilities in medicine, biochemistry, and psychology. User projects study principles for acquiring and representing symbolic domain-specific knowledge, encoding judgmental decision-making heuristics, and mechanizing inferential reasoning processes. Several working consultative programs are available for specialized areas of medical diagnosis and molecular structure analysis that can construct reasoned, explainable solutions to problems rivaling human performance. SUMEX-AIM provides a national community of user projects with hardware, software, and communication tools tailored for developing AI programs and, to a limited extent, for testing them in appropriate clinical and research settings. Available tools include list-processing languages, messaging facilities for remote collaborations and interuser contacts, and generalized AI program structures adaptable for new applications.
- *Current Research:* Modeling of diagnosis in internal medicine (CADUCEUS, formerly INTERNIST); computer-assisted consultation for cancer chemotherapy (ONCOCIN); intelligent computer-aided instruction in medical domains (GUIDON/NEOMYCIN); inference of medical knowledge from patient records (RADIX); hypothesis formation and problem solving in molecular genetics (MOLGEN); computer generation and evaluation of biomolecular synthesis pathways (SECS); modeling of human cognition and language comprehension processes (CLIPR, SOLVER); and generalization of tools for developing AI application programs (AGE, EMYCIN).
- *New Concept/Capability:* SUMEX-AIM has demonstrated the usefulness of digital communication facilities as a vehicle for scientific collaborations, software sharing, and dissemination of complex programs.

Community projects have successfully developed research versions of programs that can equal expert human performance in some specialized areas of medical diagnosis (INTERNIST, MYCIN, ONCOCIN, PUFF) and molecular structure analysis/synthesis (DENDRAL, SECS). Core research efforts and user projects

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Services Available

- *Access:* Nationally, via the commercial TYMNET and the DoD ARPANET; and locally, via phone lines and direct lines, and the Stanford University Ethernet network.
- *Service Personnel:* Systems analysts for communication and program troubleshooting; users run own jobs.
- *User Laboratory Space:* User space generally not available.
- *User Contact Persons:*

—*Prospective Users:* Lawrence M. Fagan, Ph.D., M.D., (415) 497-6979.

—*Existing Users:* Patricia L. McCabe (415) 497-5141.

—*Facility:* Edward C. Pattermann, director, (415) 497-5569.

—*Direct Inquiries to:* SUMEX Computer Project-TB105, Stanford University Medical Center, Stanford, California 94305 (415) 497-5141.

Research Emphasis or Application

have developed several examples of general program structures that can be adapted to a range of AI applications by developing appropriate knowledge bases and domain-specific rules (AGE, EMYCIN, ACT).

[1]Duda, R. O., and Shortliffe, E. H., Expert systems research. *Science* 220:261-268, 1983.
[2]Barr, A., Feigenbaum, E. A., and Cohen, P., (eds.): *The Handbook of Artificial Intelligence*, William Kaufmann, Inc., Los Altos, California, Vols. I, II, and III, 1981-82.
[3]Kunz, J. C., Shortliffe, E. H., Buchanan, B. G., and Feigenbaum, E. A., Computer-assisted decision-making in medicine. Heuristic Programming Project Report No. HPP-83-23, April 1983. *Journal of Philosophy and Medicine.*
[4]Buchanan, B. G., and Duda, R. O., Principles of rule-based expert systems. Heuristic Programming Project Report No. HPP-82-14, April 1982. (Yovits, M., ed.). *Advances in Computers*, vol. 22, New York, Academic Press.

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Title of Resource and Principal Investigator

Resource:
Support for Physiology and Biophysics Computer

Principal Investigator:
Theodore H. Kehl, Ph.D.
University of Washington
G414 HSB SJ-40
Seattle, Washington 98195
(206) 543-0943

Services Available

- *Hardware:* Laboratory automation systems designed and built by resource staff include: computer with laboratory apparatus interface circuitry, timers, analog-to-digital and digital-to-analog converters, video graphics display, hardcopy printer, and magnetic disk and tape storage options. These systems are being used in physiology laboratories at several medical schools. Design and development support equipment includes: electronics fabrication equipment, logic analyzers, oscilloscopes, programmer for PROM, PLA, and PAL.
- *Software:* Single-language computer system: Basic System Implementation Language (BASIL) and a scientific data base subsystem (NWAR) employing a tailored combination of custom and general purpose program modules that facilitate data acquisition, reduction, and analysis. General purpose program modules exist for tasks such as: analog tape recorder emulation, plotting, curve-fitting, and descriptive statistics. Custom modules have been developed for experimental control and data acquisition for a variety of physiological experiments.
- *Service Personnel:* Facility personnel maintain computers, develop new hardware and system software, and consult on user applications.
- *User Laboratory Space:* No user space provided.
- *User Contact Persons:* Theodore H. Kehl, Ph.D., (206) 543-0943. Charles R. Garthwaite, Ph.D., (206) 543-0943.

Research Emphasis or Application

- *Data Achievable with Resource's Methodologies:* Resource provides design of general purpose mini- and microcomputer laboratory automation systems with special purpose hardware/software and interfaces to physiological and biophysical experimentation. Special requirements of interactive, real-time biomedical laboratories are analyzed for desirable hardware/software solutions. Designs based on this analysis are fabricated and installed as a total system. Compilers, text editors, utilities, storage management, and user programs are written in a locally developed high-level language, BASIL. Users and system developers thus share a common programming environment. A federated processor (FP) system comprised of a collection of cooperating processing units using both private and shared resources supports a new generation of BASIL laboratory automation systems. The resource is developing the ability to design and utilize very large-scale integrated (VLSI) circuits custom-tailored to especially demanding biomedical applications.
- *Current Research:* Collaborators using the resource are actively investigating synaptic input to brain stem respiratory neurons; cutaneous sensory dorsal horn neurons; neural genesis of respiratory rhythm; time-resolved single-cell laser spectroscopy; muscle contraction; membrane biophysics; cellular aspects of cardiac defibrillation; neurophysiology; quantitative video intensification microscopy; cardiovascular control; and microvasculature. The new generation federated processor architecture BASIL system will be available to upgrade these systems. New collaborators are introduced to the system through an intensive training course conducted in Seattle.
- *New Concept/Capability: Federated Processor Architecture*—The capability of microelectronic technology continues to increase at reduced cost. To maintain low-per-unit costs, development expenses must be spread over broad markets that are not provided by biomedical laboratory automation and laboratory applications. Broader markets are stimulating electronic technology advances, which are incorporated by the modular design of the federated processor (FP) architecture; *Integrated Circuit Design and Development*—Custom integrated circuits designed for particular applications will be increasingly important

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Research Emphasis or Application

in future electronic systems. Because of market reasons mentioned above, biomedical research applications may not receive adequate consideration from commercial concerns designing new integrated circuits. The principal investigator of the resource has worked through the department of computer science to form the UW/Northwest Very Large-Scale Integration (VLSI) Consortium, a cooperative venture of the University of Washington and several industrial electronics firms. The consortium is developing tools for design of integrated circuits. The resource and its collaborators hope to design custom integrated circuits and incorporate them into BASIL system federated processors for demanding biomedical research applications such as real-time online electrophysiological waveform discrimination.

[1]Fernandez, S. M., Time-resolved fluorescence spectroscopy. In *Fast Methods in Physical Biochemistry and Cell Biology.* (Shâ afi, R.I., and Fernandez, S. M., eds) Amsterdam: Elsevier/ North-Holland, 1982.

[2]Jones, J. L., and Jones, R. E., Determination of safety factor for defibrillator waveforms in cultured heart cells. *American Journal of Physiology* 242:H657, 1982.

[3]Kehl, T. H., A filtering "any-angle" DRC/ERC (design rule checker/electrical rule checker). In University of Washington computer science department technical report 82-11-01 entitled: Northwest VLSI Consortium Semi-Annual Technical Report No. 1, 1982.

[4]Wyss, C. R., Bennett, T. D., Scher, A. M., Beat-by-beat control of cardiac output in awake dogs with atrioventricular block. *American Journal of Physiology: Heart and Circulatory Physiology* 242:H1118-H1121, 1982.

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